Antidotal Effect of Datura stramonium on the Toxicity of Carbofuran and Malathion Insecticides in Rats

By

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DEDICATION

This thesis is dedicated to

my father, who taught me, that the best kind of,

knowledge to have is that which is learned for its

own sake

To...

my mother, who taught me that even the largest

task can be accomplished if it is done

one step at a time, and who have

always instilling in me the

confidence that I am capable

of doing anything I put my mind to.

To…

my Great husband, Hashim

who gave me, the courage and support.

To…

my Precious children Ahmed

Algamri and Vivian

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Zuhour

ABSTRACT

The Antidotal Effect of *Datura stramonium* on the Toxicity of Carbofuran and Malathion Insecticides in Rats

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The purpose of the present study was to determine the antidotal effect of *Datura stramonium* (aqueous seed extract) (DSE) in the treatment of toxicity of two types of pesticides: carbofuran and malathion.

In the first experiment, (DSE) was used as a pretreatment to carbofuran toxicity compared to a reference drug (Atropine sulphate).It was studied into twenty-five albino rats arranged into five groups. The first Group dosed carbofuran 10 mg/kg. The second, third and fourth groups received 5, 7.5mg /kg of (DSE) and Atropine sulphate of 17mg/kg respectively. After the administration of (DSE) and the drug, in the second, third and fourth groups they received carbofuran 10 mg/kg body weight. The fifth group was a control.

The second experiment was to determine the antidotal effect of (DSE) in treatment of malathion toxicity. It was studied into twenty-five albino rats arranged into five groups. Four groups (group one, two, three and four) received 500mg/kg body weight of Malthion. After the administration of malathion in the four groups they received 5, 7.5mg /kg of (DSE) and 17mg/kg of Atropine sulphate respectively. The fifth group was a control.

In the two experiments, blood sample was collected for assay of serum enzymes and metabolic indicators. The rats were sacrificed after twenty-four hours. Tissue specimens were processed for histopathology.

The result of histopathology and biochemical in both experiments showed normal internal organs and normal levels of enzymes in the groups dosed 7.5 mg/kg of (DSE) and the drug. The groups received 5mg /kg of (DSE) showed mild toxicity in the internal organ and slightly high levels of serum enzymes. The groups dosed the two insecticides only showed typical toxicity in the internal organs and high level of serum enzymes. This reveals that the (DSE)

of 7.5 mg/kg has almost the same effect of the drug in treatment of both toxicities and indicates that the protective action of (DSE) against the two insecticides tested was dose dependant.

The third experiment was designed to estimate the safety of higher doses of (DSE). It was studied into sixteen white rats arranged into four groups. Three groups received 7.5, 15 and 30 mg/kg of (DSE) respectively. The fourth was a control. The rats were sacrificed after twenty four hours. Blood sample was collected for analysis of serum enzymes and metabolic indicators. Tissues were processed for histopathology. The rats dosed 15and 30 mg/kg of (DSE) showed typical toxicity in the internal organs with elevated level of serum enzymes. Rats received 7.5 mg/kg of (DSE) showed normal internal organs and normal levels of serum enzymes. These suggest that increasing the dose of (DSE) above the 7.5mg/ kg could be toxic to animals and indicate the safety of (DSE) 7.5 mg /kg dose in treatment of insecticides toxicity.

بسم الله الرحمن الرحيم

قال تعالى:(وقل ربي زدني علماً)

ملخص الأطروحة

الأثر الترياقي للداتورا سترامونيم على سمية الكاربوفيوران والملاثيون في الفئران

اعداد: زهور يحي شمس الدين الشافعي

صُممت هذه الدراسة لتحديد الأثر الترياقي لمستخلص نبات الداتورا (السيكران) في معالجة التسمم لنوعين من المبيدات هما كاربوفيوران والملاثيون .

جُرع المستخلص المائي في التجربة الأولى قبل إحداث سمية بالكاربوفيوران واستعملت سلفات الأتروبين كدواء مرجعي.اجريت التجربة على خمسة وعشرين فأراً أبيضا وزعت علي خمس مجموعات. جرعت المجموعة الأولى فموياً بالكاربو فيوران فقط. حقنت المجموعات الثانية والثالثة والرابعة بجرعتين5 و7.5ملغ/كجم من المستخلص النباتي و بجرعة17 ملغ/كجم من سلفات الأتروبين على التوالي ثم جرعت بعدها بالكاربوفيوران.استخدمت المجموعة الخامسة كشاهد.

صُممت التجربةالثانية لتقييم استعمال نفس المستخلص لعلاج سمية المالثيون. أجريت التجربة على خمسة وعشرين فأرأبيض وزعت على خمس مجموعات.جُرعت أربع مجموعات (الأولى, الثانية,الثالثة والرابعة) بجرعة500ملغ/كغم ملاثيون. حقنت المجموعات الثانية ,الثالثة والرابعة بعد تجريع المبيد بجرعتين من المستخلص5ملغ و7.5ملغ/كجم و17ملغ/كجم من سلفات الأتروبين. استخدمت المجموعة الخامسة كشاهد.

أُعدمت الفئران بعد أربع وعشرين ساعة. أخذت عينات من الدم لكشف تركيزالأنزيمات المصلية والمؤشرات الأيضية وجمعت عينات أنسجة للفحص النسيجي الدقيق الذى أوضح أن الأعضاء الداخلية للمجموعات المعالجة بالمستخلص بجرعة7.5ملغ/كجم وسلفات الأتروبين طبيعية في التجربتين وكذلك مستوي الإنزيمات.أظهرت المجموعة التي عولجت بالمستخلص بجرعة 5 ملغ/كجم ارتفاع طفيف في

مستوى الإنزيمات ووجود سمية في الأعضاء الداخلية. أوضحت المجموعات التي جرعـت بالمبيـدين ولم تُعالج ارتفاعاً في مستوى الإنزيمات المصلية مع سمية واضحة في الأعضاء الداخلية. هذه النتـائج تثبت أن المستخلص النباتي بالجرعة7.5ملغ/كجم له تقريبا نفس أثرسـلفات الأتـروبين لعـلاج سـمية المبيدين. كما يؤكذ أن علاج سمية المبيدين بالمستخلص النباتي يعتمد على الجرعة.

صممت التجربةالثالثة لتحديد مأمونية المستخلص بجرعات أعلى. درست هذه التجربة في ستة عشر فأراً أبيضاً وزعت على أربع مجموعات. حقنت ثلاث مجموعات بثلاث جرعات مـن المسـتخلص 15،7.5و30 ملجم/كجم. المجموعة الرابعة استخدمت كشاهد. ذبحت الفئران بعد اربع وعشرين ساعة حلل المصل لقياس الانزيمات. أخذت عينات أنسجة للفحص النسيجي الدقيق الذي أثبت وجود سـمية واضحة في الأعضـاء الداخليـة فـي المجمـوعتين (15و30 ملجـم/كجـم) وارتفاع في مستوى تركيزالأنزيمات المصلية مع تفاوت في الشدة. المجموعة التي حقنت بجرعة 7.5 ملجـم/كجـم كانت أعضاءها الداخلية طبيعية وكذلك تركيز الانزيمات. هذا يؤكد أن الجرعة5. 7 ملجم/كجم آمنة للاستعمال في الحيوان وأي جرعة أعلى منها قد تشكل خطراً عليه.

INTRODUCTION

Plants have always played a major role in the treatment of animals' traumas and diseases worldwide. They have been used as sources of modern drugs, either by providing pure compounds, starting materials for partial synthesis of useful compounds or models for synthesis of new drugs.

The reasons for the frequent use of traditional medicine being: the strong association of people with local flora and their belief on traditional knowledge regarding plants as medicine, easy availability of local medicinal plants, relatively poor access to synthetic drugs and their high cost, plus lower economic profile of the people.

According to the World Health Organization (WHO 1978a), 80% of the world population uses medicinal plants in the treatment of diseases and in African countries, this rate are much higher. WHO has developed the first global WHO Traditional Medicine Strategy in 2002 (WHO 2002-2005). For their importance Sudan has developed a system to regulate and register these medicinal plants through National Medicines and Poisons Board.

In all countries of the world, there exists traditional knowledge related to the health of humans and animals. According to the World Health Organization (WHO 1978a), the definition of traditional medicine may be summarized as the sum total of all the knowledge and practice, whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing.

Traditional medicine might also be considered as a solid amalgamation of dynamic medical known-how and ancestral experience. In Africa, traditional healers and remedies made from plants play an important role in the health of

millions of people. In Sudan, traditional medicine is closely related to religion an d other powerful beliefs

There are many traditional healers throughout the country but they are mainly found in the central states, especially in the Gezira area, (Ahmed *et al* 1999). The relative ratios of traditional practitioners and university trained doctors in relation to the whole population in African countries are revealing. In Ghana, for example, for every traditional practitioner there are 200 people, against one university trained doctor for nearly 20,000 people. The same applies to Swaziland where the ratios are for every traditional healer there are 100 people while for every university trained doctor there are 10,000 people (WHO, 1984), The WHO has called for recognition of traditional healers, and in 1978 declared at Alma Ata that African traditional healers should be part of the primary health care team (WHO, 1978a).

Folk medicines including herbal medicines and teas, in which the active compounds of the plants are directly used, are the most commonly used of medicinal plants. The use of pure active compounds, obtained from medicinal plants, is also widely used. The Sudanese have inherited a rich knowledge on medicinal plants and a wide some of Sudanese Medicinal Culinary and Aromatic Plants are used in folk medicines. *cassia senna* (known locally as sannamaka) grows in North, West and middle of Sudan. The fruits are used as purgatives. *Solenostemma argel* (known locally as Hargel) grows in Northern Sudan and is used as antispasmodic.*Haplophylum tuberculat* (known as Haza) grows in Northern Sudan and is used as antidiarrheal. Sudanese women also traditionally utilize fragrances in the fumigant sauna. These mainly consist of either *Acacia seyal* (known as Shaf) or *Terminalia brownii* (known as Talih) this sauna is also used for medicinal purposes (e.g. rheumatism). There are many other plants which are used as food, flavoring, perfumes and other medicinal purposes (Mofida, 2003**)**.

The overall effect of any herbal scientific study is to establish or confirm the credibility of the use of that herb or medicinal plants as an effective source of both traditional and modern medicine, and that what has been attempted to investigate in this study

The objectives of this study were:

-To investigate the use of *D.stramonium* seed extract in treatment of insecticide toxicity.

-To determine the safety use of *D.stramonium* seed extract as an antidote to poisoning in correlation with doses and the possibility of increasing the chosen dose.

CHAPTER ONE

LITERATURE REVIEW

Sudan has a very unique geographical position. This is reflected in its diverse climate and its varied natural resources. This makes suitable for cultivation and growth of many plants. Thus, the flora of the Sudan consists of 3137 species of flowering plants belonging to 170 family and 1280 genera. Of these, 278 species and 210 genera and 72 families have already been identified and Sudanese Medicinal Culinary and Aromatic Plants Research is expected to identify others **(**Elamin, 1990; Elghazali, 1986; Elghazali, *et al*, 1987; 1994; 1997).

The increasing interest in medicinal plants promoted the scientist all over the world to investigate their biological use since these medicinal plants are essential for treatment of many important diseases where other cures have yet to be found.

1.1. Plant used in this experiment: *Datura stramonium*

1.1.1Taxonomy:

Kingdom *Plantae*- plants
Subkingdom *Tracheobionta*-- vascular plants
Division *Magnoliophyta* - flowering plants, angiosperms
Class *Magnoliopsida*- dicots, dicotyledons
Subclass *Asteridae*
Order *Solanales*
Family *Solanaceae*—nightshades

Genus *Datura*

Spesies *Datura stramonium*

1.1.2. Common Names:

Jimson Weed is a common name of *Datura stramonium* which belongs to the family *Solanaceae*. The plant is native to Asia, but is also found in the West Indies, Canada, and the United States. Of the more than 12 species, *Datura stramonium* is the most common type found in the eastern United States.(Ellenhorn, 1988) Many names have been given to this plant including: Jimson Weed, Locoweed, Angel's Trumpet, Thorn Apple, Devil's Trumpet, Mad Apple, Stink Weed, Sacred Datura, Green Dragon, and Devil's Trumpet. (Ellenhorn, 1988, Goldfrank, 1994). In Sudan it is locally named(Alsikran)

1.1.3 Habitat:

Datura stramonium grows in dry waste ground and amongst rubble or the ruins of old buildings (Livingstone, 1978, Chiej, 1984 Hadad and Winchester 1990). It grows common weed along roadsides, in cornfields and pastures, and in waste areas (Ellenhorn, 1988). The plant prefers light (sandy) and medium (loamy) soils and requires well-drained soil. The plant prefers neutral and basic (alkaline) soils. It cannot grow in the shade. It requires dry or moist soil.

In Sudan, *D. stramonium* is widely distributed at Kassala, Dongla, Barber, Nuba mountains and Khartoum Province (Khadiga 1995)

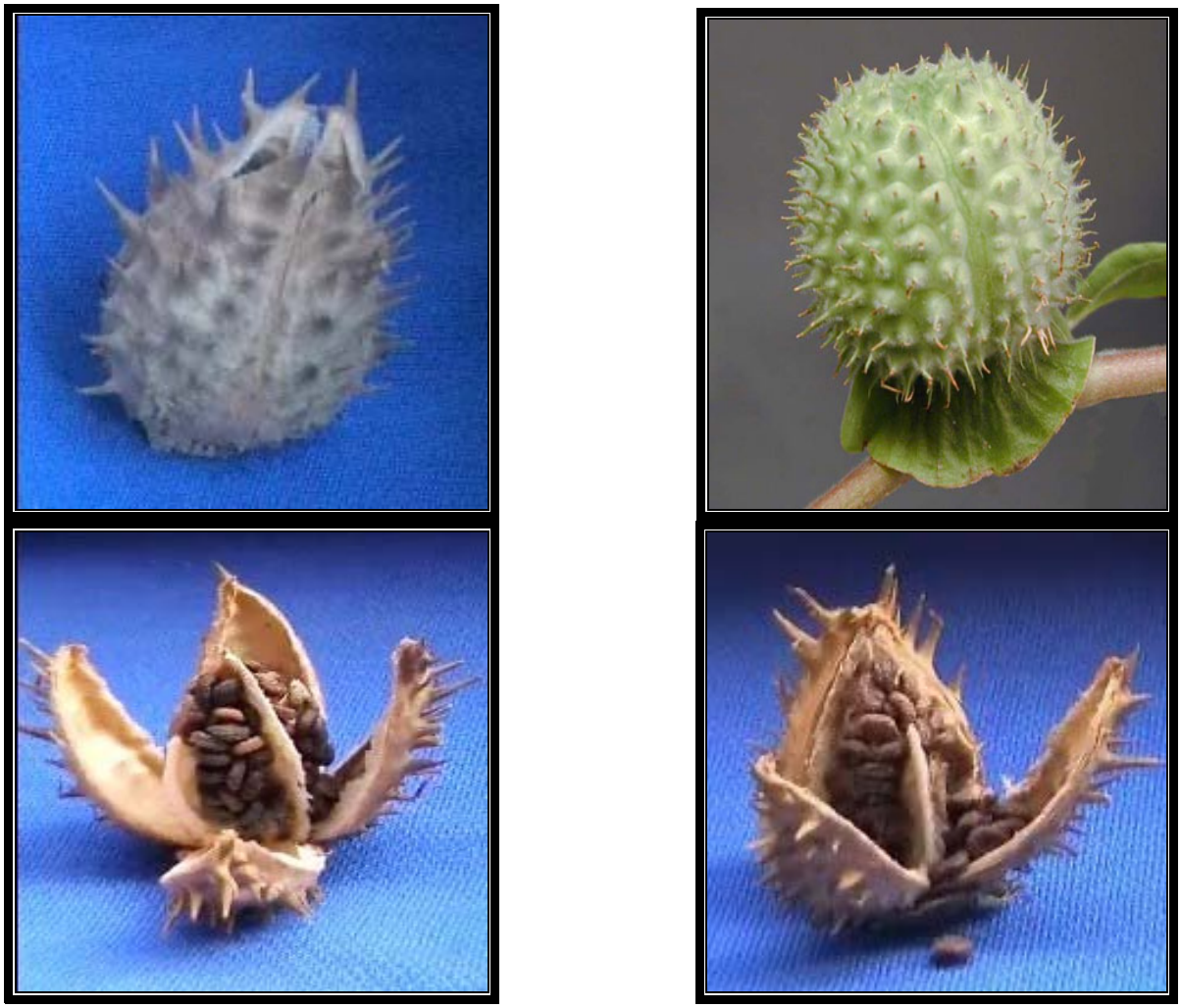
1.1.4. Description of the plant:

Datura stramonium(FIg1, 2 and 3) is an annual plant. It grows 4 to 6 feet tall and has dark green, long stemmed, lobed leaves that exude a foul odor. Its flower that four lobed, thorny, green seed pod fruit ripens in early falls. Each lobe contains 50-100, 2-3 mm, kidney-shaped, black blooms in late spring is usually white, sometimes lavender, solitary and tubular brown seeds. (Ellenhorn 1988, Goldfrank1994)

Fig (1) ***Datura stramonium*** **natural growth**

Fig (2): *Datura setramonium* fruit

A B



D **c**

A- ***D. stramonium*** ripe fruit

B -*.D. stramonium* green fruit

C and D- Four segmented lobes of ***D.stramonium*** containing ripe seeds.

Fig (3) *Daura stramonium* seeds

It is in flower from July to October, and the seeds ripen from August to October. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Moths.

1.1.5. History and folklore:

The origin *of D. stramonium* is disputed (Curtain, 1947). The Sanskrit dhattura and the Hindustani dhatur formed the basis of the general name, the origin of Jimson weed could be Asiatic. Some sources report a probable Central American origin, due to *D.stramonium* habitation of most temperate and subtropical parts of the world. "The native names applied by ethnic groups appear to be based upon the deliriant effects produced by the plant on the nervous system" (Bye *et al* 1991).Throughout the ages, the Devil's Trumpet has been used for both intoxication and as medicine.

The plant has been described throughout history as a toxin famous for its mind-altering properties. There are references to it in Homer's Odyssey, and Shakespeare's plays: Hamlet, Romeo and Juliet, and Anthony and Cleopatra. **(**Ellenhorn, 1988)**.**

It is one of a group of plants known as "belladonnas," thought to be named from their use by Italian women to dilate their pupils, which was considered beautiful. **(**Gilman 1990) Its most common name, Jimson Weed, is a contracted form of Jamestown Weed, after its use was described in 1676, in Jamestown, Virginia. Jimsonweed is named for a case of human poisoning in Jamestown, Virginia, when British troops sent to halt Bacon's Rebellion; soldiers were poisoned by eating the plant in a salad and then suffered delirium and hallucinations. (Goldfrank ,1994).

In some tribes *D. stramonium* was involved in the ceremonies of manhood. The sadhus(is a common term for a mystic, an ascetic, practitioner of yoga

(yogi) and/or wandering monks) of Hinduism also used *D. stramonium* as a spiritual tool, smoking it with cannabis in their traditional chillums. It was also widely used by the Magyar (Hungarian) spiritual leaders (the Táltos) since ancient times (Ratsch 2005)

1.1.6 Active Compounds and pharmacology

The toxic principles are tropane belladonna alkaloids which possess strong anticholinergic properties (Ruhwald, 2005). These alkaloids include, hyoscyamine (stems, leaves, roots, seeds), hyoscine (roots); atropine (d,l-hyoscyamine) and scopolamine (l-hyoscine) (Miraldi, *et al*, 2001,. Steenkamp, *et al*,2004) All parts of the plant are toxic, but the greatest amount of alkaloids is contained in ripe seeds (Day and Dilworth 1984, Dugan *et al* 1989 , Boumba, *et al* 2004, Ruhwald 2005). Young, dried leaves (Naude, *et al*, 2005) and stems and leaves of young plants (Miraldi, *et al*, 2001)

They act as competitive antagonists to acetylcholine at peripheral and central muscarinic receptors at a common binding site. (Gilman, 1990)

The peripheral receptors are on exocrine glands which affect sweating, salivation, and smooth and cardiac muscle. Poisoning results in wide-spread paralysis of parasympathetic innervated organs. (Ellenhorn, 1988).

As tertiary amines they also have central nervous system absorption, inhibit CNS receptors and result in a central anticholinergic syndrome of acute psychosis or delirium (**(**Haddad and Winchester, 1990**)**.

Tropane alkaloids are some of the few substances which cause true hallucinations . Scopolamine can be slowly and erratically absorbed into the brain. In most people, scopolamine reaches the brain within an hour or so after ingestion and causes visual and auditory hallucinations. Scopolamine acts as a competitive antagonist at muscarinic acetylcholine receptors, specifically M1 receptors; it is thus classified as an anticholinergic,anti-muscarinic drug. The exact concentration of specific alkaloids varies with

species, cultivation, environment, temperature, moisture, and storage. The range of toxicity is highly variable and unpredictable; toxicity may vary from leaf to leaf, plant to plant and season to season. This contributes to the danger of misuse of the plant since the dose cannot be predicted. **(**Haddad and Winchester, 1990). The highest concentration of alkaloids occurs in the seeds approximately 0.1 mg of atropine per seed or 3-6 mg/50-100 seeds. (Goldfrank, 1994). Toxicity of *D. stramonium* is induced when they are ingested, smoked and absorbed topically especially through mucous membranes.

These toxins are easily absorbed from mucous membranes and the gastrointestinal tract. The half-life of atropine is approximately 4 hours. Metabolism occurs in the liver by hydrolysis which eliminates approximately half the drug. The remainder is excreted unchanged in the urine. (Ellenhorn**,** 1988, Gilman, 1990)

It is estimated that 100 seeds contain 6 mg of atropine equivalent. (Frohne and Pfander, 1984, Palmer *et al* 2002) the authors estimated that 1 g of seeds contained 8.34 mg of atropine. Younger plants have less atropine and scopolamine content in the seed; these concentrations increase as the plant ages. (Miraldi et al 2001).

1.1.7. Clinical effects and toxicity:

The thorn apple is a bitter narcotic plant that relieves pain and encourages healing (Bown, 1995). It has a long history of use as a herbal medicine, though it is very poisonous and should be used with extreme caution.

The mnemonic for clinical effects of typical atropine poisoning is: "blind as a bat, mad as a hatter, red as a beet, hot as a hare, dry as a bone, the bowel and bladder lose their tone, and the heart runs alone." Symptoms include

mydriasis, cycloplegia, flushed, warm, dry skin; dry mouth; ileus, urinary retention, tachycardia, hyper or hypotension, delirium with hallucinations, jerky, myoclonic movements, choreoathetosis, hyperthermia, coma, respiratory arrest, rare seizures, and central stimulation followed by depression. (Haddad and Winchester, 1990) Hallucinations are reported in as many as 83% of cases, typically they are simple visual images in natural colors, but frequently also tactile hallucination of crawling insects (Ellenhorn, 1988). The onset of symptoms occurs within 30 to 60 minutes after smoking leaves or drinking tea; and 1-4 hours after ingestion of plant material or seeds (Goldfrank, 1994) Initial symptoms include dry mouth then pupil dilatation. (Ellenhorn 1988).

The duration of symptoms is often 24-48 hours because of delayed gastrointestinal motility; symptoms have been reported to last up to 1-2 weeks. Although, poisoning may lead to fatal medullary paralysis arrhythmias and Cardiovascular Collapse, (Hanna 1992).Jimson Weed-relat ed deaths mainly are as a result of impaired judgment and coordination resulting in risk-taking activities associated with accidental death. (Ellenhorn, 1988, Coremans *et al* 1994) All Animals species can be poisoned. The literature mentions poisoning of cattle, goats, horses, poultry, sheep, and swine. Because of the plant's strong odor and unpleasant taste, animals consume it only when other food is not available. The seeds are sometimes milled with other seeds and have caused problems Jimsonweed can be harvested with hay or silage, and subsequently poisoning occurs upon feeding the forage. Seeds can contaminate grains and is the most common poisoning that occurs in chickens. (Cooper and Johnson, 1984, Cheeke and Schull 1985, Lampe and McCann, 1985).

1.1.8. Medicinal Uses:

It has a long history of use as herbal medicine, though it is very poisonous and should be used with extreme caution. The leaves, flowering tops and seeds are anodyne, antiasthmatic, antispasmodic, hallucinogenic, hypnotic, mydriatic and narcotic (Chiej, 1984, Launert, 1981, Bown, 1995)

D. stramonium is now used to treat asthma, and gastrointestinal problems, also aches, abscesses, arthritis, boils, headaches, hemorrhoids, rattlesnake bites, sprains, swellings, and tumors (Sandoval, 1998). It acts as a sedative in large doses and as a stimulant and deleriant in high doses. *D. stramonium* is an anodyne, antibiotic, antispasmodic and narcotic. Relieving the pains of rheumatism and sciatica when applied as an ointment, and easing spasms of Parkinsons disease are unproven accounts of the effects of Jimson weed. An ointment of the ground seeds and suet is rubbed on boils, pimples, and swellings; the powdered leaves are applied to hemorrhoids; and hot baths containing the plant give relief to colds and diarrhea (Curtain, 1947)

Most of the plant is used for medicinal reasons. Eating the seeds rapidly gets the plant to the nervous system, but also increases the risk of lethal overdose. The leaves can be dried and smoked to relax the bronchiole muscles of the throat, and leaves are used also to line beds of those with insomnia. (Sandoval, 1998) recommends using the fresh leaves, flowers, or seeds. In an infusion, 2 teaspoons of fresh leaves per cup of hot water, or a poultice using any variation of the recommended parts.

The thornapple is a bitter narcotic plant that relieves pain and encourages healing **(**Bown, 1995).

The leaves can be used as a very powerful mind-altering drug, they contain Hyoscyamine and Atropin, and there are also traces of scopolamine, a potent

cholinergic-blocking hallucinogen, which has been used to calm schizoid patients (Weiner, 1980).Atropine dilates the pupils and is used in eye surgery (Goldfrank ,1994).

The leaves have been smoked as an antispasmodic in the treatment for asthma, though this practice is extremely dangerous (Weiner, 1980, Foster and Duke, 1990). Also as analgesic, anthelmintic and anti-inflammatory, they are used in the The leaves have been smoked as an antispasmodic in the treatment for asthma, though this practice is extremely dangerous (Weiner, 1980, Foster and Duke, 1990). Also as analgesic, anthelmintic and anti-inflammatory, they are used in the treatment of stomach and intestinal pain due to worm infestation, toothache and fever from inflammations (Tsarong, 1994). Externally, it is used as a poultice or washes in the treatment of fistulas, abscesses wounds and severe neuralgia (Bown, 1995, Moerman, 1998) seeds are used in Tibetan medicine, they are said to have a bitter and acrid taste with a cooling and very poisonous potency (Tsarong, 1994**)** It should be used with extreme caution and only under the supervision of a qualified practitioner since all parts of the plant are very poisonous and the difference between a medicinal dose and a toxic dose is very small (Lust, 1983, Weiner, 1980, Bown, 1995). The antidote of choice for poisoning is physostigmine. The growing plant is said to protect neighboring plants from insects (Riotte, 1978)

Treatment of patients following an organophosphate (OP) exposure can deplete a hospital's entire supply of atropine. Given the possibility of multiple severe exposures after a terrorist attack OP nerve agents; there exists a need for either greater atropine stores or the using development of alternative antidotes. Jimson weed (*D. stramonium)* contains atropine and other anticholinergic compounds and is common and readily available (Theodore *et al*, 2004)

Jimson weed extract was used as a protective agent in severe Organophosphate toxicity; by (Theodore, *et al* 2004). They have proved that pretreatment with Jimson weed extract significantly increases survival following severe dichlorvos exposure in white r**ats.**

1.2. Pesticides:

The use of organophosphate and carbamate pesticides for pest control on or around animals has increased. In general, the organophosphate and carbamate pesticides are more toxic to warm-blooded animals there were increased incidences of pesticide poisonings among domestic and agricultural animals, it is necessary to recognize the signs of pesticide poisoning in animals and initiate prompt treatment when poisoning is suspected.

1.2.1 Malathion:

Malathion is a general use pesticide. It is available in emulsifiable concentrate, wettable powder, dustable powder, and ultra low volume liquid formulations

Malathion is one of the most commonly usedOrganophosphates, it has a wide range of uses including agriculture, stored grain and forests. It is used to control a large variety of pests, including mosquitoes, grasshoppers, gypsy moths, Mediterranean fruit flies, mites, aphids, spider mites, and scales.(Brenner, 1992) It is applied via ground and aerial sprays, aerosols, foggers, baits, paints, pet collars, animal dips, animal dust bags, and cattle feed blocks.

1.2.1.1. Mode of action

Malathion works by attacking the nervous system; it is essentially a nerve poison. Specifically, it inhibits the action of an enzyme called acetylcholinesterase (AChE). (WHO, 1986, U.S. E.P.A, 2000). AChE works

to break down another chemical, acetylcholine, which is essential in transmitting impulses between nerves. Therefore, when malathion is used, AChE becomes unable to break down acetylcholine, which consequently accumulates in nerve cells. This abnormal acetylcholine build-up can cause in coordination, rapid twitching, in coordination, paralysis and death. (Tucker and Thompson, 1987, Gallo and Lawryk, 1991).

Malathion's toxicity is increased by its break-down products and contaminating chemicals. For instance, as malathion reacts and breaks down within an organism or in sunlight, one of the chemicals released is malaoxon. Malaxon is 40 times more toxic than malathion, and is the primary source of malathion's toxicity. (Borwn *et al*, 1993). In addition, there are 11 chemicals that are created in the malathion production process which can often be found in malathion.

1.2.1.2 Toxicity of Malathion:

Malathion is slightly toxic via the oral route, with reported oral LD50 values of 1000 mg/kg to greater than 10, 000 mg/kg in the rat, and 400 mg/kg to greater than 4000 mg/kg in the mouse. In another study, it is estimated that Ld 50 in rats is 390-480 mg/kg (Hazleton and Holland, 1953)

It is also slightly toxic via the dermal route, with reported dermal LD50 values of greater than 4000 mg/kg in rats effects of malathion are similar to those observed with other organophosphates, except that larger doses are required to produce them. It has been reported that single doses of malathion may affect immune system response. (Gallo, and Lawryk, 1991)

Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoardination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and

slow heartbeat.Very high doses may result in unconsciousness, incontinence, and convulsions or fatality. The acute effects of malathion depend on product purity and the route of exposure other factors which may influence the observed toxicity of malathion include the amount of protein in the diet and gender. As protein intake decreased, malathion was increasingly toxic to the rats (Carlson, 1987)

Malathion has been shown to have different toxicities in male and female rats and humans due to metabolism, storage, and excretion differences between the sexes, with females being much more susceptible than males (Menzer, 1987).

Rats fed dietary doses of 5 mg/kg/day to 25 mg/kg/day over 2 years showed no symptoms apart from depressed cholinesterase activity. When small amounts of the compound were administered for 8 weeks, rats showed no adverse effects on whole-blood cholinesterase activity (Gallo, and Lawryk, 1991). Weanling male rats were twice as susceptible to malathion as adults

Malathion is mutagenic and carcinogenic (Cantor, *et al*. 1992.)Has been implicated in vision loss, kidney damage (Albright, *et al.* 1983) .It has been shown to cause DNA abnormalities (Balaji and Sasikala, 1993). A 2002 study by scientists from Assan and North-Eastern Hill Universities (India) showed that malathion given orally caused genetic damage in laboratory mice (Giri, *et a*l. 2002). Another 2002 study, from Egypt's National Research Center, showed that mice fed with stored wheat that had been treated with a commercial malathion insecticide developed two kinds of genetic damage. The damage occurred at all dose levels tested in this study (Amer, *et al* 2002)

The pesticide has been shown in animal testing and from use experience to affect the central nervous system, immune system, adrenal glands, liver, and

blood. Recent research also shows that malathion has a variety of harmful effects on birds. Malathion disrupted normal hormone activity, caused genetic damage, and reduced birds' food supply (George *et al*, 1995). It is considered moderate to very high in toxicity to fish and other water organisms. A study in (India) showed that a concentration of 50 ppb of malathion damaged red blood cells in the common fish *Channa punctatus*. (Sawhney and Johal, 2000)

1.2.1.3. Fate in animals:

Malathion is rapidly and effectively absorbed by practically all routes including the gastrointestinal tract, skin, mucous membranes, and lungs. malathion undergoes similar detoxification mechanisms to other organophosphates, but it can

also be rendered nontoxic via another simple mechanism, splitting of either of the carboxy ester linkages. Animal studies indicate it is very rapidly eliminated though urine, feces and expired air with a reported half-life of approximately 8 hours in rats and approximately 2 days in cows (Gallo and Lawryk, 1991)

1.2.2. Carbamate pesticides- carbofuran:

Carbofuran is a carbamate pesticide used to control a broad spectrum of insects on corn, rice, Alfa Alfa, grapes, and other foodstuffs. It is sprayed directly onto soil and plants just after emergence to control beetles, nematodes, and rootworm (U.S. 1EPA, 1995). It is available in liquid and granular formulations.

Carbofuran was evaluated for an acceptable daily intake in 1978 and 1979 and a temporary ADI for man was estimated to be 0-0.003 mg/kg body weight

(WHO and FAO 1980). The available data reflected that carbofuran is a highly toxic carbamate ester whose metabolic profile has been well defined.

1.2.2.1 Mode of action:

Metabolism of carbofuran has been evaluated in rats, mice, insects, and plants. It appears that the main route consists of oxidation at the benzylic carbon to yield hydroxycarbofuran, which can then be hydrolyzed to 3-hydroxycarbofuran phenol and 3-ketofuran-7-phenol. Another pathway of metabolism, which is more common in mammals than insects and plants, is to hydrolyze carbofuran directly to the carbofuran phenol (Metcalf, *et al* 1968, U.S. EPA, 1990).The anticholinesterase properties of the three major carbofuran metabolites were evaluated; particularly those with intact carbamyl moieties. The moieties, 3-hydroxycarbofuran, 3-ketocarbofuran, and 3-hydroxy -N-hydroxy methylcarbofuran were found to have less anticholinesterase activity than carbofuran itself (Dorough, 1968; 1983).

1.2.2.2Toxicity of Carbofuran:

Exposure to carbofuran causes a rapidly reversible direct cholinesterase inhibition. As with other carbamate compounds, carbofuran's cholinesterase-inhibiting effect is short-term and reversible (Kearney, and Kaufman 1975).

The influence of carbofuran metabolism on acetylcholinesterase inhibition has been defined after low dose (50 µg/kg, i/v and oral) carbofuran exposures to male Sprague–Dawley Rats, showed that Red blood cell acetyl cholinesterase (RBC AchE) inhibition was 83% at 2 min, 37% at 15 min for i/v and oral, respectively,

with recovery by 3 hours and correlated with carbofuran plasma concentrations (Ferguson *et al* 1984).

The lethal effects of carbofuran are due largely to the chemical's direct inhibition of acetyl cholinesterase. Ultimate cause of death is respiratory failure. Signs and symptoms of cholinesterase poisoning occur within minutes as carbofuran acts directly on the enzyme without metabolic activation (Tobin, 1970).

Symptoms of carbofuran poisoning include: vomiting, abdominal cramps, sweating, diarrhea, excessive salivation, weakness, imbalance, blurring of vision, breathing difficulty, increased blood pressure, and incontinence. Death may result at high doses from respiratory system failure associated with carbofuran exposure (Baron, 1991).Clinical signs of anticholinesterase poisoning were seen in two treated groups of rats, consisting of tremors and teeth grinding (Ellman *et al*., 1961)

Complete recovery from an acute poisoning by carbofuran, with no long-term health effects, is possible if exposure ceases and the victim has time to regain their normal level of cholinesterase and to recover from symptoms. The oral LD50 is 5 to 13 mg/kg in rats, 2 mg/kg in mice, and 19 mg/kg in dogs. The dermal LD50 is >1000 mg/kg in rabbits, (Baron, 1991). The LC50 (4-hour) for inhalation of carbofuran is 0.043 to 0.053 mg/L in guinea pigs; (Kidd and James, 1991).

An evaluation was made on the possible differences in brain and erythrocyte cholinesterase activity in adult and juvenile rats of both sexes following acute exposure to carbofuran. Examination of the optimal sampling time for evaluating maximum erythrocyte cholinesterase depression showed that approximately 30 minute following acute administration was the optimal in both sexes in adults and juveniles. The data showed recovery was almost complete four hours following acute carbofuran treatment. Complete recovery was noted at the 24-hour interval. It was quite evident that complete recovery of all enzyme depression was attained within one day (Case, 1980).

Chronic toxicity was recorded in carbofuran administered to rats caused a significant decrease in water consumption as well as in brain, serum and erythrocyte cholinesterase activities. Statistically significant increases in relation to the control were found in the serum enzyme activities (Dragica *et al*, 2008).

1.2.2.3. Fate in animals:

Administered carbofuran is rapidly excreted. After 60 minutes, 6 % and 24 % of the dose from labeled carbofuran was detected in the exhaled breath and urine of mice, respectively. (Ahdaya *et al,* 1981) found that after eight hours of dermal administration, 72 percent of the dose was eliminated with approximately 2/3 in the feces and 1/3 in the urine of mice. The dose excreted by feces was approximately half of the administered dose. (Shah *et al*, 1981)

1.3 Atropine:

Atropine is the best-known member of a group of drugs known as muscarinic antagonists, which are competitive antagonists of acetylcholine at muscarinic receptors. This naturally occurring tertiary amine was first isolated from the *Atropa belladonna* plant by (Weiner, 1985) although atropine earlier enjoyed widespread use in the treatment of peptic ulcer, today it is mostly used in resuscitation, anaesthesia, and ophthalmology, usually as the more soluble sulphate salt. By competitively blocking the action of acetylcholine at muscarinic receptors, atropine may act as a specific antidote.

Atropine is usually prepared by extraction from the plants *Atropa belladonna* (deadly nightshade), *D. stramonium* (Jimson weed) or *Duboisia myoporoides* (McEvoy, 2002). This extracted atropine is a combination of D and L hyoscyamine, both these isomers may bind to muscarinic receptors (Berghem

et al, 1980), although the pharmacological activity is thought to be due almost entirely to L hyoscyamine (McEvoy, 2002).

Atropine sulphate occurs as odorless, very bitter, colorless crystals or white crystalline powder (Budavari, 1996, Parfitt, 1999). The alkaloid, atropine, is

an organic ester which may be prepared synthetically by combining tropine and tropic acid (McEvoy 2002), but is usually obtained by extraction from some solanaceous plants (Parfitt, 1999).

1.3.1 Mode of antidotal activity

Atropine is a muscarinic cholinergic blocking agent. It competitively blocks parasympathetic, postganglionic nerve endings from the action of acetylcholine and other muscarinic agonists. Atropinic drugs have little effect at nicotinic receptor sites. Large doses of atropine produce only partial block of autonomic ganglia and have almost no effect at the neuromuscular junction.

The peripheral antimuscarinic effects of atropine may not be the only antidotal property of the drug in organophosphate poisoning. Atropine may also be of value in treating acute dystonic reactions occasionally observed in acute organophosphate poisoning (Smith *et al* 1979; Joubert *et al*., 1984; Joubert and Joubert, 1988; Wedin, 1988). Patients with extrapyramidal signs have been noted to have abnormally low plasma and red blood cell cholinesterase activities, producing an excess of acetylcholine relative to dopamine (Wedin, 1988). However, there is little clinical evidence available on the possible anticonvulsive effects of atropine in man.

It is a specific antidote for the treatment of poisoning with organophosphorus and carbamate insecticides the role of atropine in the treatment of

organophosphate poisoning is essentially unchallenged, though there is controversy concerning the dose of atropine necessary for optimal therapy in organophosphate poisoning. (Schoene et al, 1988) studied the efficacy of Atropine sulphate administered prior to dosing with paraoxon in mice.

Atropine sulphate was administered 5, 20, 40 and 60 minutes before organophosphate administration and was found to reduce mortality significantly.

In calves poisoned with intravenous Dichlorvos, atropine was shown to reverse the respiratory effects of the organophosphate *(*Likeux *et al*, 1986). Atropine may reverse changes in ventilation-perfusion inequalities resulting from uneven distribution of ventilation; caused by acetylcholine-mediated airway constriction (Slocombs and Robinson, 1987).Support for an anticonvulsant action of Atropine has been presented by (McDonough *et al,* 1987) who found that Atropine pre-treatment prevented the development of convulsions and brain damage induced by soman or VX injected directly into the amygdala.

1.3.2 Pharmacokinetics:

Atropine is absorbed irregularly from the gastrointestinal tract, and more slowly than with parenteral dosing (Dollery, 1991). Intramuscular absorption of Atropine and Atropine sulphate depends on the method of injection, the site of injection (Friedl *et al*, 1989). Atropine and atropine sulphate reach peak plasma levels when injected intramuscularly in about 30 minutes (Berghem *et al*, 1980, Saarnivaara *et a*l, 1985, Gervais *et a*l, 1997).

Atropine is metabolized in the liver by microsomal monooxygenases. HPLC separation of urine has identified 5 compounds: Atropine, Noratropine, Tropine, Atropine-N-oxide (equatorial isomer), and tropic acid. Thus,

Atropine is partly metabolized and partly excreted unchanged in the urine, the unchanged portion being approximately 50% (Van der Meer et al, 1983, 1986). Intramuscular Atropine may slow small bowel transit time by approximately 38% (Hardison *et al*, 1979). Gastric emptying may also be delayed by atropine (Dollery, 1991).

Atropine is the drug of choice for the treatment of the muscarinic symptoms and signs of poisoning with anticholinesterase agents (organophosphate or carbamate**,** particularly excessive salivation and lacrimation, bronchoconstriction and hypersecretion, pulmonary oedema and bradycardia. Animal studies have shown that Atropine alone significantly reduces mortality and counteracts features of toxicity due to most anticholinesterase insecticides. In several clinical case reports the administration of Atropine is clearly associated with a reduction of cholinergic features and a favorable outcome. (Andrew, 2000)

CHAPTER TWO
MATERIALS AND METHODS

2.1. Materials and experimental designs:

Three experiments were run to determine the use of herbal antidote to the toxicity of some pesticides. In the first experiments the aqueous extract of *D.stramonium* seeds was used as pretreatment to toxicity induced by a carbamate (carbofuran), compared to reference drug Atropine sulphate. In the second experiment, the same extract was used as post treatment to toxicity induced by an organophosphorus (malathion).The third experiment was run to determine the safety of different levels of doses, of the aqueous seed extract of the plant.

2.1.1 Pretreatment of carbofuran induced toxicity with *D.stramonium* aqueous extract:

2.1.1.1 Animals, housing and management:

Twenty five, male and female white (Albino) rats of weight 115-180 gm, were obtained from the Medicinal and Aromatic Plant Research Institute, National Centre for Research, Khartoum, Sudan on 23/1/2008. They were housed under standard environmental conditions, within the premises of the Medicinal and Aromatic Plant Research Institute. They were housed in cages in a room at controlled temperature (22± 2 ºc), relative humidity 60% with free access to water and formula rat feed (2.5 meal and 20% protein).Animals were apparently healthy and they were identified by tail color marks relevant to their groups. One week was allowed as preliminary adaptive period.

2.1.1.2 Administration and dose rates:

At the end of the adaptation period, the animals were weight-distributed and allotted randomly to five groups, each of five rats. Group1 rats were treated orally with carbofuran 10 mg/kg dissolved in normal saline. Group 2 and 3 were pretreated with *D.stramonium* seed aqueous extract (5 and 7.5 mg /kg body weight respectively dissolved in normal saline I/p, after 5 min each group was treated orally with carbofuran 10 mg/kg body weight.
Group 4 rats were pretreated with Atropine sulphate 17 mg/kg body weight I/p. After 5 min they received carbofuran 10mg/kg. Group 5 served as unintoxicated untreated control.

2.1.1.3 Parameters:

Clinical signs were observed. Blood samples were harvested from the ocular vein. The blood samples were collected successively three hours post administration of carbofuran , six hours and twenty four hours. Blood samples were collected into dry clean bottles. Sera were analyzed for the concentration of cholinesterase and the activities of ALT, AST, and ALP and the concentrations of metabolic indicators, total protein, Albumin, globulin, urea and creatinine. The rats were scarified after twenty four hours, tissue specimens of liver, heart, and kidney. Spleen and brain were fixed in 10% buffered formalin and processed for histopathology.

2.1.2 Treatment of malathion induced toxicity in rats with *D.stramonium* seed extract:

2.1.2.1 Animals, housing and management:

Twenty five, male and female white (Albino) rats of weight 115-180 gm, were obtained from the Medicinal and Aromatic Plant Research Institute,

National Centre for Research, Khartoum, Sudan on 23/1/2008. They were housed under standard environmental conditions, within the premises of the Medicinal and Aromatic Plant Research Institute. They were housed in cages in a room at controlled temperature (22± 2 ºc), relative humidity 60% with free access to water and formula rat feed (2.5 meal and 20% protein).Animals were apparently healthy and they were identified by tail color marks relevant to their groups. One week was allowed as preliminary adaptive period.

2.1.2.2 Administration and dose rates:

At the end of the adaptation period, the animals were weight- distributed and allotted randomly to five groups each of five rats.

Group 1 received only malathion 500 mg/ kg/ body weight. Group 2 was exposed to Malathion 500 mg/kg /body weight and after half an hour treated with *D. stramonium* seed extract 5mg /kg/body weight, intraperitonially (I.P). The treatment dose was repeated after four hours. Group 3, received malathion 500 mg/ kg /body weight and after half an hour treated with *D. stramonium* seeds extracts at a dose of 7.5mg/kg /body weight I/P. The treatment dose was repeated after four hours. Group 4, were exposed to toxicity by malathion 500 mg /kg/ body weight orally and post treated with Atropine sulphate 17 mg/kg/body weight I/.P. The Atropine sulphate dose was repeated after four hours. Group 5 served as control group (unintoxicated, untreated).

2.1.2. 3 Parameters:

Clinical signs were observed. Blood samples were harvested from the ocular vein. The blood samples were collected three times. The first blood samples were collected after half an hour from the administration *D. stramonium* seed extract and Atropine sulphate . The second; after half an hour from the second treatment (by *D. stramonium* seed extract and atropine). The third blood

samples, after twenty four hours from the second treatment (by *D. stramonium* seed extract and atropine). Sera were analyzed for the concentration of cholinesterase and for the activities of Alanine amino transferase (ALT), Aspartate amino transferase(AST) and Alkaline Phosphatase (ALP). The concentrations of metabolic indicators, total protein, Albumin, globulin, urea and creatinine were also evaluated. The rats were scarified after twenty four hours; tissue specimens of liver, heart, kidney, spleen and brain were fixed in 10% buffered formalin and processed for histopathology.

2.1.3 Toxicity of *Datura Stramonium* in white rats:

2.1.3.1 Animals, housing and management:

Twenty five, male and female white (Albino) rats of weight 115-180 gm, were obtained from the Medicinal and Aromatic Plant Research Institute, National Centre for Research, Khartoum, Sudan on 23/1/2008. They were housed under standard environmental conditions, within the premises of the Medicinal and Aromatic Plant Research Institute. They were housed in cages in a room at controlled temperature (22± 2 ºc), relative humidity 60% with free access to water and formula rat feed (2.5 meal and 20% protein).Animals were apparently healthy and they were identified by tail color marks relevant to their groups. One week was allowed as preliminary adaptive period

2.1.3.2 Administration and dose rates:

At the end of the adaptation period, the animals were weight- distributed and allotted randomly to four groups each of four rats. Group 1 received *D. stramonium* seed extract at a dose of 7.5 mg/kg intrapretonially, group2 injected by the plant extract at a dose of 15 mg/kg intrapretonially, group 3,

received the plant extract at a dose of 30 mg/kg intrapretonially. Group 4, was untreated or the control group.

2.1.13 parameters:

Clinical signs were observed. The rats were sacrificed after twenty-four hours and blood samples were collected. Sera were analyzed for the activities of ALT, AST, and ALP and for the concentrations of metabolic indicators, total protein, albumin, globulin, urea and creatinine. Tissues specimens, of liver, heart, kidney, spleen and brain, were fixed in 10% buffered formalin and processed for histopathology.

2.2. METHODS

2.2.1 The extraction method (Aqueous extract) of the plant:

D. stramonium plant collected in winter (in the middle of November2007) from Shambat area near the river Nile bank. It possessed large numbers of ripened fruits. The plant is dried at room temperature. The seeds were gathered from the thorn apples -small black seed, (the seed weighed approximately 0.01g).

The seeds weights were (78.35-gram).The coarse powder of the plant seeds were soaked in boiling distilled water and left for two hours at room temperature. The mixture was filtered. The filtrate was cooled over night at 4ºC. The concentrated extract was freeze - dried (Komolafe *et a*l, 1988). The yields percentages were calculated and the residues were kept in the refrigerator until used. The extract weight was 12. 272 gm.

2.2.2 Histological methods:

The specimens were collected immediately after slaughter and fixed in 10% buffered formal saline, embedded in paraffin wax, sectioned at 5 um and stained with heamotoxlin and eosin (H&E) using Mayere haemalum.

2.2.3 Chemical methods:

Blood samples obtained from the ocular vein of rats were used to prepare sera for the chemical methods, Venous blood samples were allowed to clot at room temperature for 30 minutes. Serum was separated by centrifugation at 3000 R.P.M for 5 minutes and stored at -20 ºc until analyzed. Spectrophotometer (Merck Mega, Version 0.6 1995 E Merck, Dramstadt, Germany) was used to record serum activities of enzymes AST, ALT and ALP and serum metabolites, albumin, total protein, urea, creatinine and cholinesterase.

2.2.3.1 Glutamyl oxaloacetic transeaminase(Aspartate amino transferase. L.Aspartate: 2-oxaglutarate amino- transferase. E C.6.1.1:G.O.T, A.S.T):

Serum AST activity was measured by commercial kit (Randox Laboratories L td U.K)

It is an enzymatic method that measures glutamic oxaloacetic transaminase in serum according to (Retiman and Frankel 1975) and (Schmidt and Schmidt, 1963).

2.2.3.1.1 Test principle:

Aspartate aminotransferase, catalyses reversible transferase, of an amino group, aspartate to a- ketoglutrate and oxaloacetate.

2.2.3.1.2 Reaction:

L-aspartate +a-oxoglutrate $\xrightarrow{\text{GOT}}$ L – Glutamate + Oxaloacetat

The oxaloacetate produced is reduced to Malate by Malate Dehydrogenase (MDH) and NFDH.

Oxalacetate +NDH + H $\xleftrightarrow{\text{MDH}}$ malate + NAD

The rate of decrease in concentration of NADH is proportional to the catalytic concentration of AST present in the serum sample.

2.2.3.1.3 Protocol:

None – Heamolysed serum was added to buffered substrate mixture of L-aspartate and a-oxaloglutrate. The absorbance at a wave length of 365 nm was read at one minute intervals after mixing with the buffered substrate solution. The mean absorbance change per minute (A365/ minute) was used for calculation of enzymes activity as follows:

IU=A365nm/miute ×2059.

2.2.3.2 Orthophosphoric mono- ester phosphydrolase E.S.3.13.1; ALP. Alkaline Phosphatase:

It optimized method according to recommendation of (Chemie 1972)

2.2.3.2.1 Test principle:

In alkaline medium serum alkaline phosphatase splits p- nitro phosphate, In the presence of Mg ions, into p- nitrophenyl and phosphate. At the PH of reaction, p- nitro phenyl was yellow. The optical density measured in spectrophotometer at wave length 405 nm.

2.2.3.2.2 Reaction:

P-nitrophenyl phosphatase + H_2O $\xrightarrow{ALP}$ inorganic Phosphate + p-nitrophenol

2.2.3.2.3 Calculations:

ALP is calculated as follows:

U/I = 2760 × A405 nm/min

(A = the mean of sample absorbance reading)

2.2.3.3 Alanine amino transferase ALT (Glutamic Pyruvic transaminase, L_ aspartate,oxoglutamate:

It is an enzymatic method, which measures glutamic pyruvic transaminase in serum according to Retimen and Frankel. (1975) and (Schmidt and Schmidt 1963).

2.2.3.3.1 Test principle:

Alanine amino transferase are measured by monitering the concentration of pyruvic hydrazone formed with 2-4 dinitrophenylhydrazine

2.2.3.3.2 Reaction:

Oxoglutarate +L _ Alanine $\xrightarrow{GPT}$ L- glutrate + pyruvic

2.2.3.3.3 Protocol:

None – Heamolysed serum was added to buffered substrate mixture of L-Alanine and a-oxaloglutrate. The absorbance of samples was read against the blank after 5 minutes at wave length of 630 nm UV/VIS, Spectrophotometer. The ALT was measured in I.U/L.

IU=A630nm/miute ×2059.

2.2.3.4 Total protein:

Total serum protein was measured by colorimetric method using a concentration kit (Randox laboratories, L td, U.K.)

2.2.3.4.1 Test Principle:

Colorimetric determination of total protein in serum is based on the Biruet reaction. The serum protein reacts with copper sulphate in the presence of sodium hydroxide. The Rochelle (K-Na- -tarterate) contained in the Biruet reagent is utilized to keep the formed cupric hydroxide in solution which gives the blue color. The intensity of the color produced is proportional to the amount of protein in the sample. The absorbencies of the sample, (A sample) and of the standard (A standard) were read against the reagent blank in the Spectrophotometer at a wave length of 545 nm. The total protein concentration (C) was calculated as follows:

$$C\ (mg/dl) = \frac{A\ sample}{A\ standard} \times \text{concentration of the standard}$$

2.2.3.5 Albumin:

Serum albumin was measured by a colorimetric method using a commercial kit (Randox laboratories Ltd.,U.K

2.2.3.5.1 Test principle:

The measurement of serum albumin is based on its quantitative binding to the indicator 5, 5-di-BCP.

Serum was mixed with a buffered BCP reagent and the mixture was incubated for 2 minutes at room temperature. The absorbance of the sample (A sample) and of the standard (A standard) was measured against the reagent blank at wave length of 600 nm and albumin concentration (C) was calculated as follows:

$$C\ (g/dl) = \frac{A\ sample}{A\ standard} \times \text{concentration of the standard}$$

2.2.3.6 Globulin:

Globulin concentration was detected by subtracting albumin concentration from total protein concentration as follows:

C (total protein) – C (albumin) = C (globulin)

2.2.3.7 Urea:

Serum urea concentration was estimated by an enzymatic colorimetric method using a kit (Randox Laboratories L td, U.K.)

2.2.3.7.1 Test principle:

Ammonia and carbon dioxide are produced when urea is hydrolyzed by urease

$$Urea + H_2O \xrightarrow{urease} Ammonium\ carbonate + CO_2$$

Ammonium ions react with phenol and hydro phenol and hydro chlorite to give a colored complex

2.2.3.7.2 Protocol:

The serum was mixed with a buffered unease solution and the mixture was incubated 10 min at room temperature. The hydrochloride solution was added and mixed and the contents were incubated at room temperature for 15 min. The absorbency of the sample (A sample) and of the standard (A standard) were read against the blank at a wave length of 546 nm and the concentration (c) of the urea was calculated as follows:

$$C\ (mg/dl) = \frac{A\ sample}{A\ standard} \times \text{concentration of the standard}$$

2.2.3.8 Creatinine:

Serum creatinine concentration was measured by a colorimetric method using a commercial kit (Randox laboratories Ltd., UK)

2.2.3.8.1 Test principal:

Creatinine forms a colored complex with picric acid in an alkaline medium. The color intensity is proportional to the concentration of the reaction mixture. Serum was mixed with a mixture of pioric acid and Sodium hydroxide. Absorbance change/ minutes of the sample and the standard were recorded at wave length of 492 and the change in absorbance was used in calculations as follow:

$$C\ (mg/dl) = \frac{A\ sample}{A\ standard} \times \text{concentration of the standard}$$

2.2.3.9 Cholinesterase:

Serum cholinesterase concentration was estimated by an enzymatic calorimetric method using a kit (Linear laboratories –Spain)

2.2.3.9.1 Principle:

Cholinesterase (CHE) catalyzes the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine. The latter reduces 5, 5´-mercaptobis-2-nitrobenzoic acid (DMNB) to 5-mercapto-2-nitrobenzoate (5-MNBA), a colored compound. The reaction is monitored kinetically at 405 nm by the rate of information of the yellow color produced, proportional to the activity of (CHE) in the sample 1.

Butyrylthiocholine + H_2O ⟶ Butyrate + Thiocholine

Tiocholine + DMNB ⟶ Oxidized thiocholine + 5-MNBA + H^+

Dibucain inhibition can be estimated by performing concurrent assays in which dibucaine is present in the substrate mixture. Percent inhibition is evaluated by comparison of activity in the inhibited system with that in the uninhibited system. The resulting dibucaine number allows the classification and identity of the homozygous and heterozygous variants.

2.2.3.9.2 Cholinesterase calculations:

The difference in absorbance was used to calculate the concentration of Cholinesterase as follow:

Total cholinesterase

U/L = ΔA/30sec x 46222 (37ºC)

U/L= ΔA405 nm/min

U/L x 16.67 = nkat/L

2.24 Statistical Method:

Data in serum enzyme and metabolites were subjected to one way analysis of variance ANOVA. Mean values were compared using un-paired student t-test. Data in serum enzyme and cholinesterase in variance treatments were drawn as histograms and compared (Snedecor and Cochram 1989)

CHAPTER THREE

RESULTS

3.1 Pretreatment of toxicity induced by carbofuran with *Datura stramonium* aqueous extract:

3.1.1 Clinical signs:

Group1 (carbofuran 10mg/kg),this group treated by carbofuran alone as intoxicated control, signs of toxicity started after 5 minutes and include tremor, restlessness and inappitance, after 1hr one rat of the group died. The signs of toxicity continued for 3 hrs**.** In group 2 (*Datura stramonium* seeds extract 5 mg /kg body weight and carbofuran10 mg/kg), the signs of toxicity started after 5 minutes and include tremor, restlessness, and inappitance. No mortalities recoded and the signs of toxicity disappeared after 1 hour and 48 min. In group 3 (*Datura stramonium* seed extract 7.5mg /kg body weight and carbofuran10 mg/kg), in this group the signs of toxicity started after 6 minutes this group showed the same clinical signs as in group 2 but less in severity. After 1 hour and 30 min. no signs were recorded. No mortalities were recorded. The rats in this group started to drink and eat normally after 2 hours. Group 4 (Atropine sulphate 17 mg /kg body weight and carbofuran 10 mg/ kg), In this group the clinical signs started after 11 minutes and they are the same in character to the previous ones but less in severity, after 1 hour and 27 min. later 3 rats behave normally, the rest of the two showed slight movement .

3.1.2 Pathological changes:

In test group 1 **(**carbofuran 10 mg/kg), the changes in the organs are characterized by sever congestion and hemorrhages in the liver, kidney and

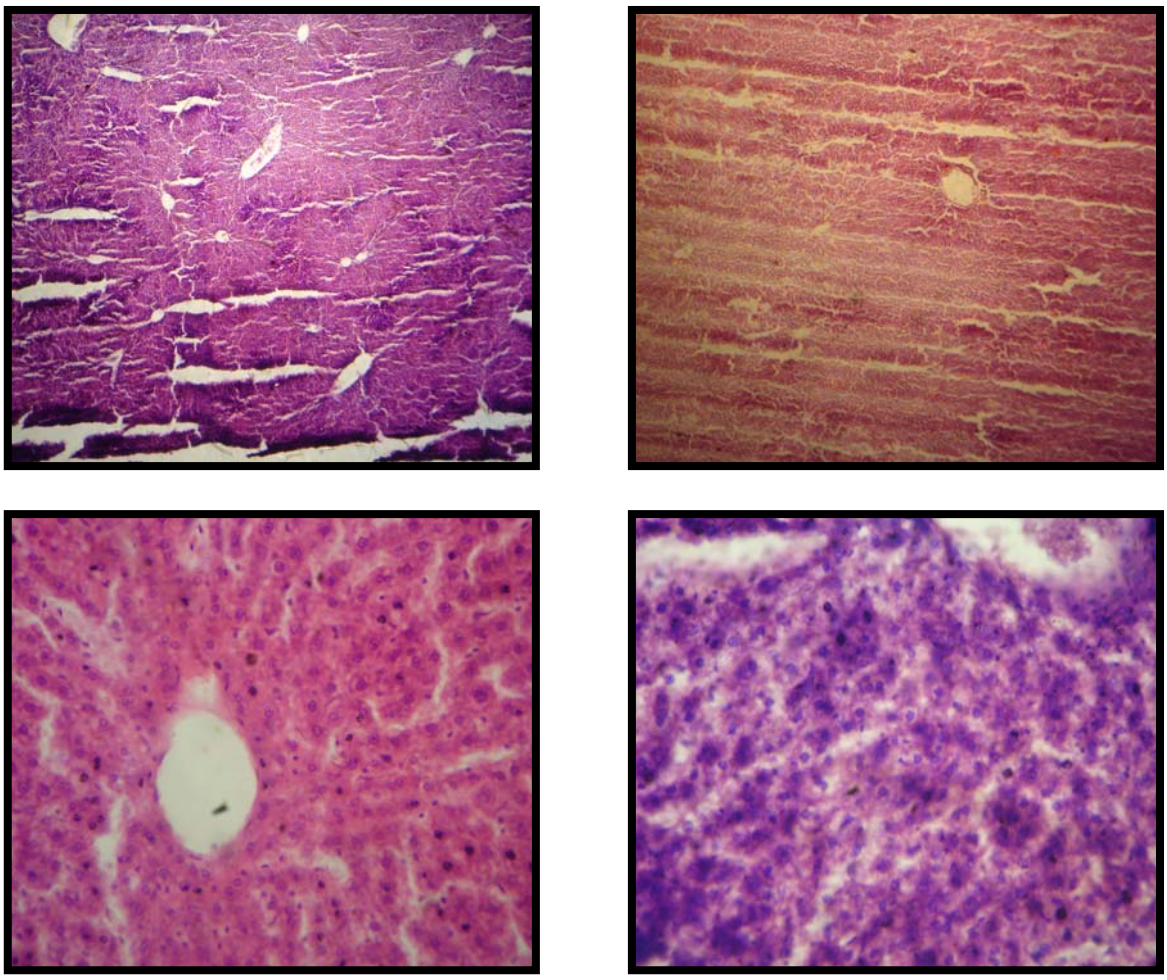
Fig (4) Congestion, hemorrhage in liver and spleen in rat treated with carbofuran

heart (Fig 4).The brain of the same group showed congestion and hemorrhages. Where as in group 2 (*D. stramonium* seed extract 5 mg/ kg and carbofuran 10 mg/kg) the changes, in the liver, heart, and kidneys were, fatty changes, congestion and hemorrhages. The test groups **(***D. stramonium* seed extract 7.5mg /kg body weight and carbofuran 10 mg/kg) and Atropine sulphate 17 mg//kg body weight and (carbofuran 10 mg/kg) had the same pathological changes fatty change in the liver, slight congestion the heart, brain and kidneys.

3.1.3 Histological changes:

Histopathological changes in livers in groups 1, 2, 3 and 4 were showen in fig 5(A-D).The liver in-group 1(carbofuran 10 mg /kg) showed centrilobular necrosis and fatty changes. In-group 2 (*D.stramonium* seed extract 10 mg/kg and carbofuran 10 mg /kg there were scattered areas of necrosis of the hepatocytes and fatty changes. In group 3 (*D.stramonium* seed extract 7.5 mg/kg and carbofuran 10 mg /kg) showed slight fatty changes and congestion. In group 4 (17 mg/kg and carbofuran 10 mg /kg) the liver showed slight fatty change. Other organs showed different pathological changes. In group1, the kidneys showed fatty changes of medudulary tubules. The brain showed severe meningitis with lymphocytic infiltration. In the heart, there was necrosis of the myocardium. In Group 2(*D. stramonium* seed extract 5mg /kg body weight and carbofuran 10 mg/kg) the heart showed mild necrosis. The same changes in the previous group were seen in the brain, kidney. Group 3 (*D. stramonium* seed extract 7.5mg /kg body weight and carbofuran10 mg/kg) showed mild necrosis in the heart, there was mild infiltration in the brain. No histopathological changes in the spleen. In group 4 (Atropine sulphate 17 mg /kg body weight and carbofuran10 mg/kg), the heart, brain and kidneys had the same histopathological changes that seen in group 3.

A **B**



C **D**

FIG (5) Sections of livers of rats treated with carbofuran , Atropine sulphate and *D. stramonium* seed extract 5 mg and 7.5 mg /kg body weight.

A- Centrilobular necrosis and fatty changes in a liver of rat dosed with carbofuran 10 mg /kg (group 1).

B- Scattered areas of necrosis of the hepatocytes and fatty changes in a liver of rat dosed with *D.stramonium* seed extract 10 mg/kg and carbofuran 10 mg /kg (group 2).

C- Slight fatty changes and congestion in a liver of rat dosed with *D.stramonium* seed extract 7.5 mg/kg and crbofuran 10 mg /kg (group 3).

D- Slight fatty change in a liver of rat dosed with Atropine sulphate 17 mg/kg and carbofuran 10 mg /kg (group **4).**

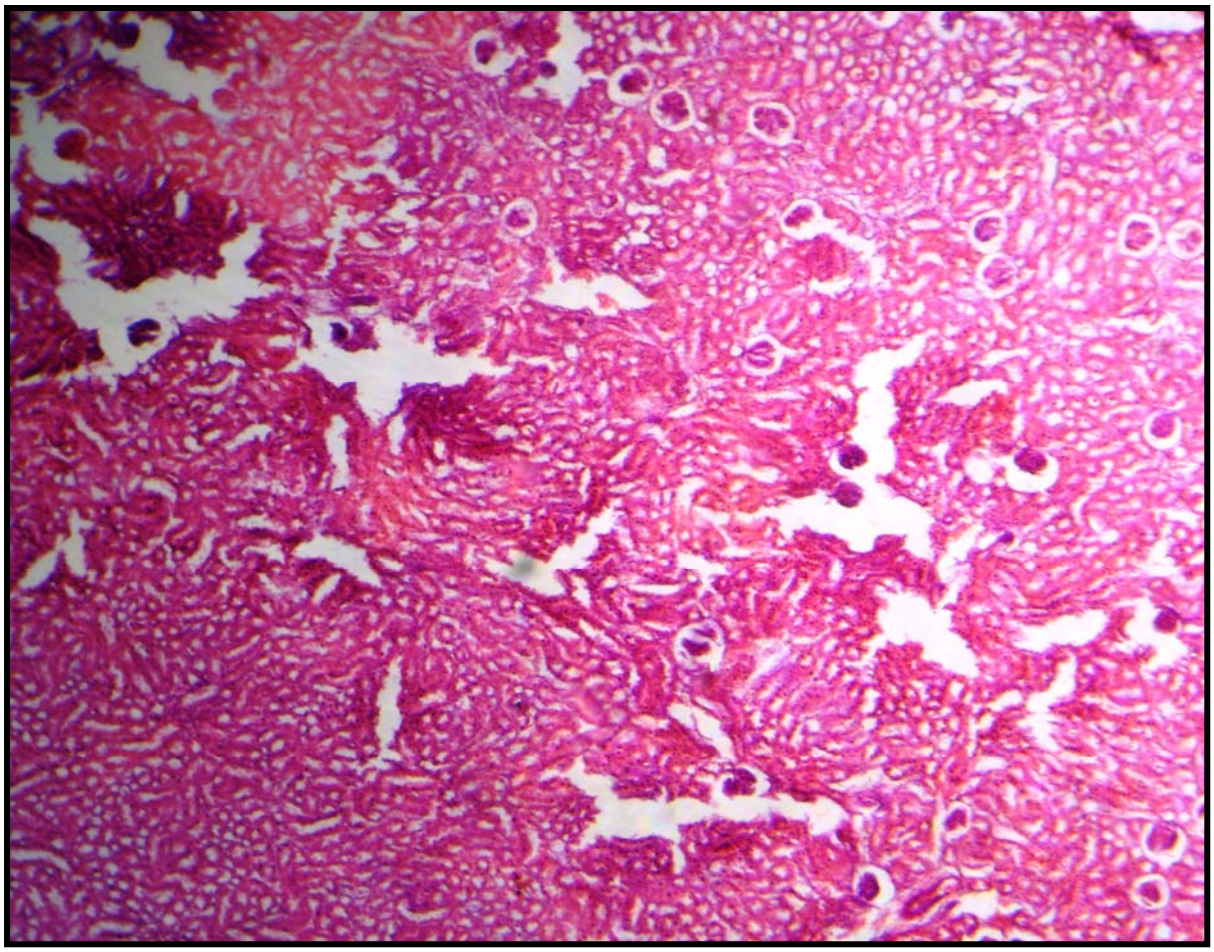


Fig (6) Focal area of necrosis and fatty change in a kidney of rat dosed carbofuran 10 mg /kg

3.1.4 Changes in the serum constituents:

Table (1) and Fig (7-10) shows the changes in serum constituents of rats pretreated with *D.stramonium* seed extract and intoxicated with carbofuran group. After three hours of administration of carbofuran the activities of cholinesterase is significantly reduced in-group 1(carbofuran) when compared to the control.

The level of ALT increased significantly in-group 1(carbofuran) and group 2(*D. stramonium* 5 mg/kg and carbofuran). The activities of ALP increased significantly in all the groups but group 1(carbofuran) had the highest level of this enzyme. The activities of AST, increased significantly in group 1(carbofuran).The test groups 3 and 4 (*D. stramonium* 7.5 mg/kg and carbofuran and atropine group and carbofuran), show almost normal levels of these enzymes. Concentration of urea is increased in group1 (carbofuran), group 2(*D. stramonium* 5 mg/kg and carbofuran) and group 3 (Atropine 17 mg/kg and carbofuran).No significant increase in creatinine level. Total protein, albumin and globulin showed no significant changes when compared to the control.

After six hours after administration of carbofuran, levels of cholinesterase was increased in all the groups but it was significantly reduced when compared to the control group especially group 1(carbofuran). ALT and ALP began to decrease in all groups but the higher levels of these enzymes remain in group 1 (carbofuran) and group 2 (*D. stramonium* 5 mg/kg).AST began to decrease but group 1(carbofuran) still had a high level of this enzyme. Total protein is slightly decreased in group 1 (carbofuran) and group 2 *D.stramonium* 5 mg/kg). There were no significant change in urea concentration .The level of creatinine was significantly increased in group 1(carbofuran).

After twenty –four hour after administration of carbofuran; the level of ALT and AST were slightly increased in group1 (carbofuran). ALP activity was

Table (1) Analysis of variance and average (mean ±SE) values of serum constituents of rats pre-treated with *Datura stramonium* seed aqueous extracts and intoxicated with carbofuran:

After three hours from the first treatment dose.

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/d)l	Globulin(g/d)l	Albumin(g/d)l	Urea (mg/dl)	Creatinine(mg/dl)	Cholinesterase(nkal)t
F value**	12.7*	2.97*	5.98	2.18*	3.72 *.	1.05	0.73*	*15.35	0.54
G 1	45.50±4 a	232.50±0.5 a	155.00±13.8 a	5.72±0.3 b	3.233±0.2 a	2.575±0.3 a	56.00±2.4 b	0.525±0.0 bc	389.7±104 b
G 2	42.00±1.9a	175.00±8 b	142.50±10.2 bc	6.20±0.12 ab	3.03±0.2 a	3.10±0.0.7 a	45.2.60±3.1b	0.350±0.1 c	282.00±123.3 b
G3	28.00±4.3c	153.60±2.2 b	112.50±8.5 bc	6.22±0.1 ab	2.92±0.0 a	2.70±0.1 a	30.00±0.5 a	0.60±0.1 ab	615.33±35.3 a
G4	29.50 ±9bc	171.40 ±0.8b	121.20±10.1 b	6.02±0.1 ab	2.74±0.2 a	3.40±0.2 a	42.20±1.7 a	0.400±0.0 bc	654.33±74.1a
G 5	26.66±6.5c	162.00 ±1.3b	89.00±5.4 c	6.47±0.2a	2.83±0.0a	3.63±0.0a	33.00±0.9 a	0.75±0.0 a	766.0±12 a

After six hours

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/d) l	Globulin(g/dl)	Albumin(g/d) l	Urea (mg/dl)	Creatinine(mg/dl)	Cholinesterase(nkal)t
F value**	6.24*	7.82*	8.29*	3.89*	96*	0.22	8.94	4.99*	4.51*
G 1	36.00 ±7.6a	195.50±1.4 a	113.25±8.7 a	5.57±0.3 c	2.97 ±0.2a	2.60±0.3 a	33.00±2.4 a	0.95 ±0a	492.67 ± 104b
G 2	32.50±16.1b	178.50±2.5 b	126.33±2 ab	6.55±0.2 a	3.35±0.2a	3.20±0.1 a	30.66±2.5 a	0.47 ±0.1bc	600.33±123 a
G3	28.60±9.8 c	165.80±3.2 b	84.40±8.5 c	5.84±0.2 bc	2.88±0 b	2.96±0.1 a	31.20±3.3a	0.44±0.1 c	695.33 ±35.4a
G4	30.20±2 c	162.40±1.9 b	87.40 ±10.6 c	6.16±0.1 abc	2.88±0.3 b	3.0±0.1 a	34.0±2.3 a	0.62±0.0 b	700.33±74 a
G 5	26.25±7.6 c	147.75 ±1.5b	85.00±6.8 c	6.37±0.2 ab	3.425±0.2 a	3.17±0.1 a	36.00±0.6 a	0.63 ±0b	711.00±12 a

After twenty four hours

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/d) l	Globulin(g/dl)	Albumin(g/d)l	Urea(mg/dl)	Creatinine(mg/dl)	Cholinesterase(nkal)t
F value**	5.81	5.97*	24.67*	3.17*	3.10*	3.4*	5	*1.55	2.51
G 1	30.25±2.4 a	176±2.2 a	109.0 ±6a	3.20±1b	2.65±0.1 b	1.55±0.2d	37.0±2a	0.2 ±0.1b	634.00±98 ab
G 2	28.00± 2.7a b	149.6 ±1.6a	100.0±2.5 a	5.00±0.2 ab	3.15±0.1 a	3.15±0.1 ab	30.25 a	0.8±0.1 a	646.00±31 ab
G3	26.20±14 b	171.0±3.9 a	85.0 ± 10.3 b	5.44±1.4 a	2.94±0.1 ab	2.74±1 c	32.0 ±3.1a	0.44 ±0.1ab	690.00±19.5 a
G4	27.00±7.8 b	156.0±1.8a	90.2 ±0.2b	6.06 ±0.2a	3.24±0.1 a	2.92±0 bc	35.0±1.7 a	0.62±0 ab	700.0±59 a
G 5	25.25±5.4 b	155.0 ±2.2a	90.0±4.5 b	6.62±0.2 a	3.15±0.2 a	3.4±0.7 a	35.0 ±0.0a	0.6±0.1 ab	710.00±33.5 a

G1 = (Carbofuran10mg/kg).
G2= (Datura seed extract 5mg /kg body weight and Carbofuran10 mg/kg:
G3= (Datura seed extract 7.5mg /kg body weight and Carbofuran10 mg/kg
G4= (Atropine 17 mg /kg body weight and Carbofuran10 mg/kg G5= (control).

Means with the same letter are not significantly different (.P> 0.05)

*At (4, 20) degrees of freedom. ** 5%

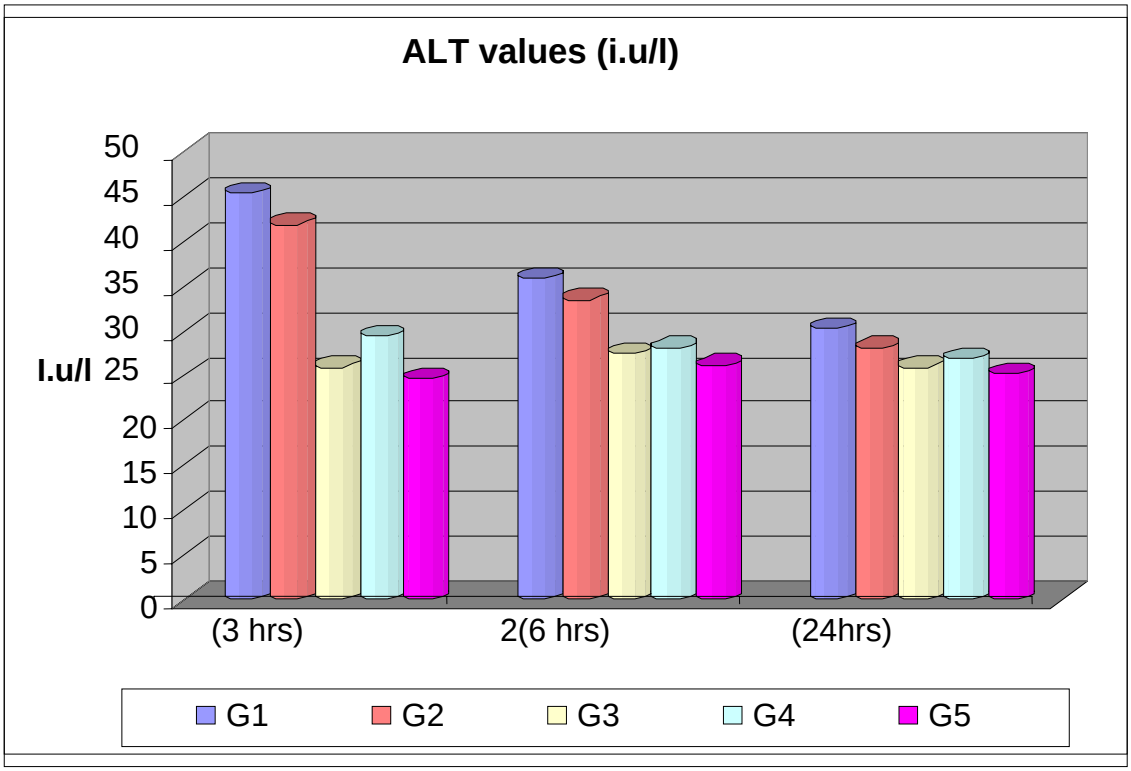


Fig. (7) Comparison between ALT levels in rats treated with carbofuran, *Datura Stramonium* seed extract and carbofuran and Atropine sulphate and carbofuran:

G1 = (carbofuran 10mg/kg)

G2= (D.Stramonium seed extract 5mg /kg body weight and carbofuran 10 mg/kg)

G3= (D. Stramonium seed extract 7.5mg /kg body weight and carbofuran 10 mg/ kg)

G4= (Atropine sulphate 17 mg /kg body weight and carbofuran 10 mg/ kg)

G5= (control)

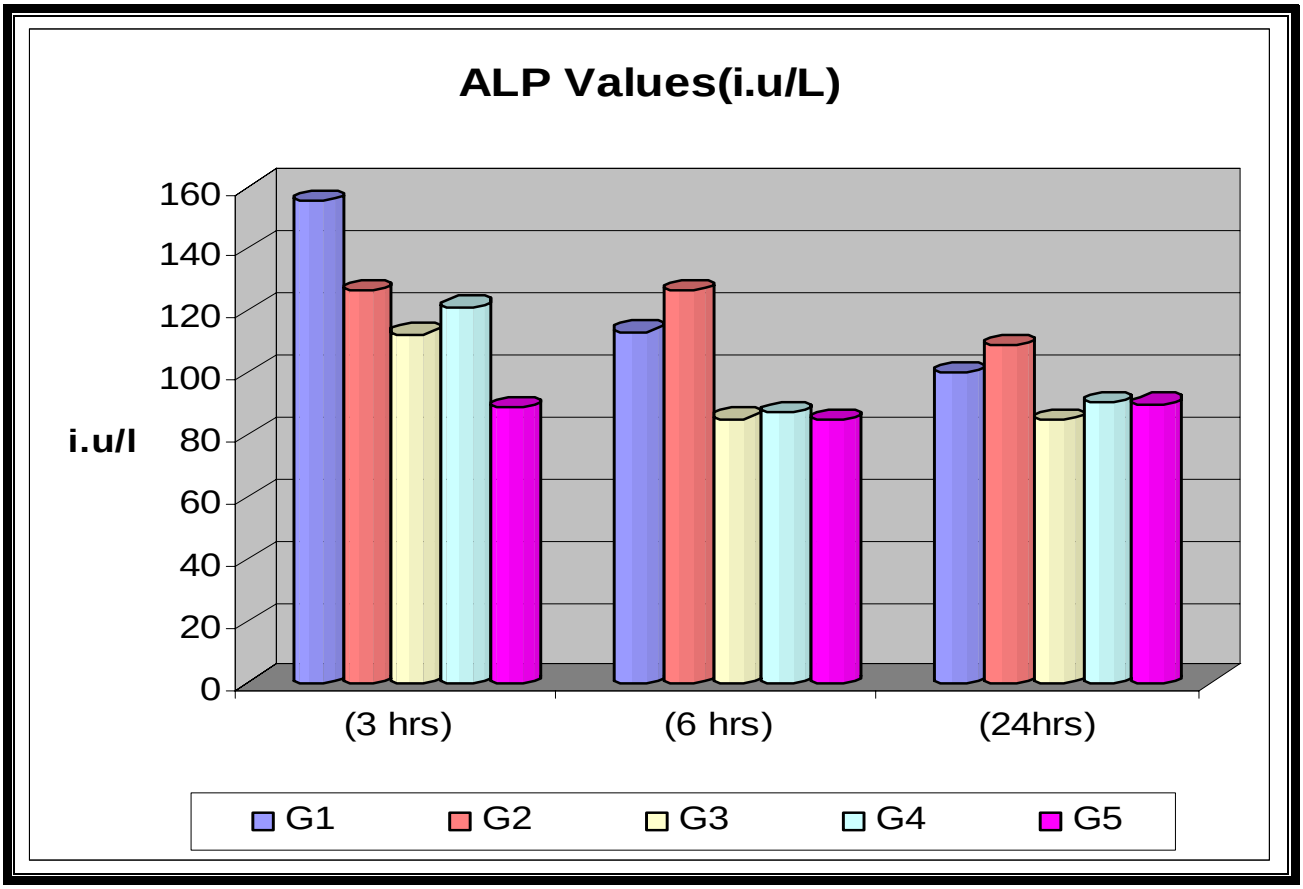


Fig (8) Comparison between ALP levels in And rats treated with carbofuran, *Datura Stramonium* seed extract and carbofuran and Atropine sulphate and carbofuran

G1 = (carbofuran10mg/kg):

G2= (D. stramonium seed extract 5mg /kg body weight and carbofuran 10 mg/kg)

G3= (D. stramonium seed extract 7.5mg /kg body weight and carbofuran 10 mg/kg)

G4= (Atropine sulphate 17 mg /kg body weight and carbofuran 10 mg/kg)

G5= (control)

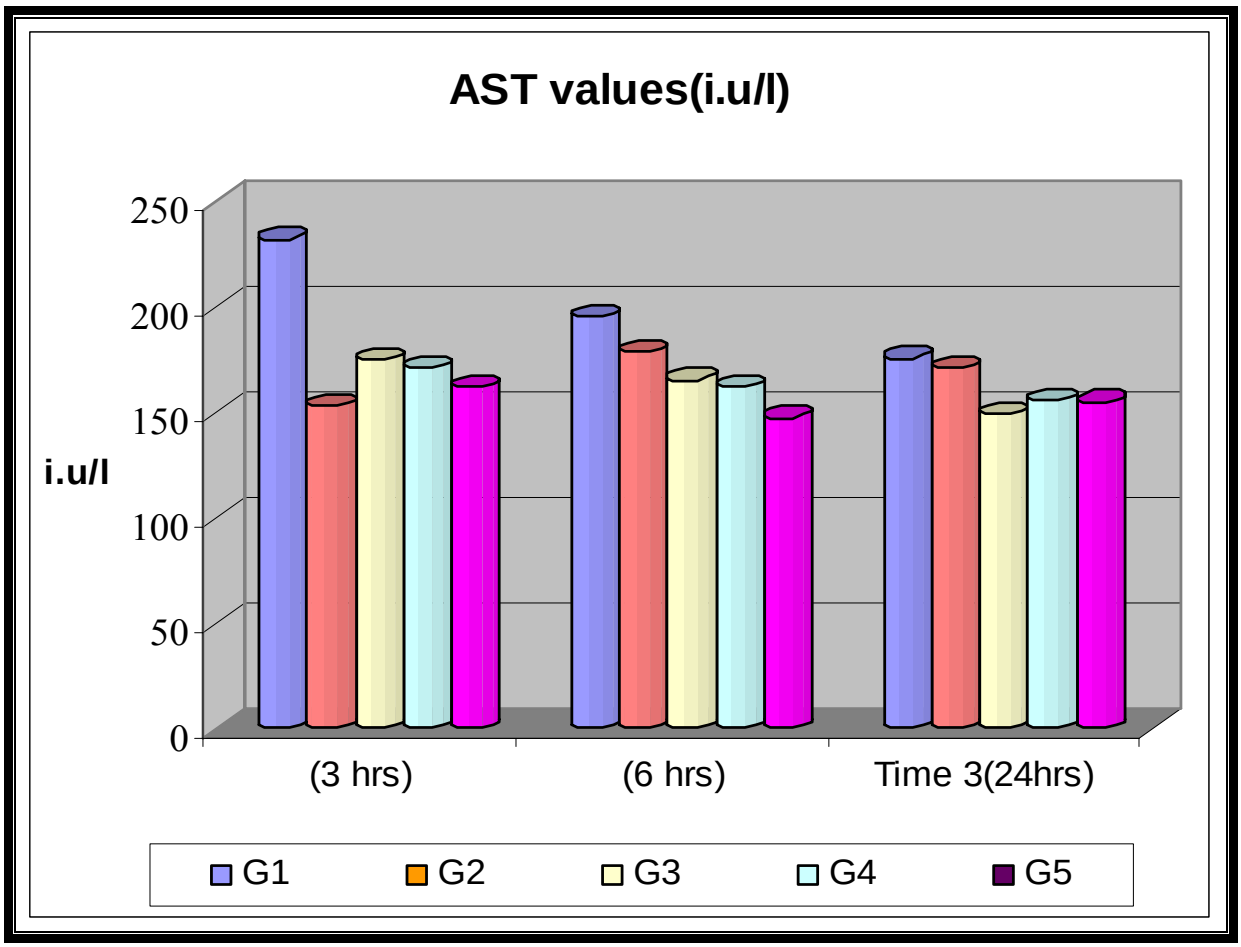


Fig (9) Comparison between AST levels in rats treated with carbofuran, *Datura Stramonium* seed extract and carbofuran and Atropine sulphate and carbofuran

G1 = (Carbofuran10mg/kg)

G2= (D. stramonium seed extract 5mg /kg body weight and carbofuran10 mg/kg)

G3= (D. stramonium seed extract 7.5 mg /kg body weight and carbofuran10 mg/kg)

G4= (Atropine sulphate 17 mg /kg body weight and carbofuran10 mg/kg)

G5= (control)

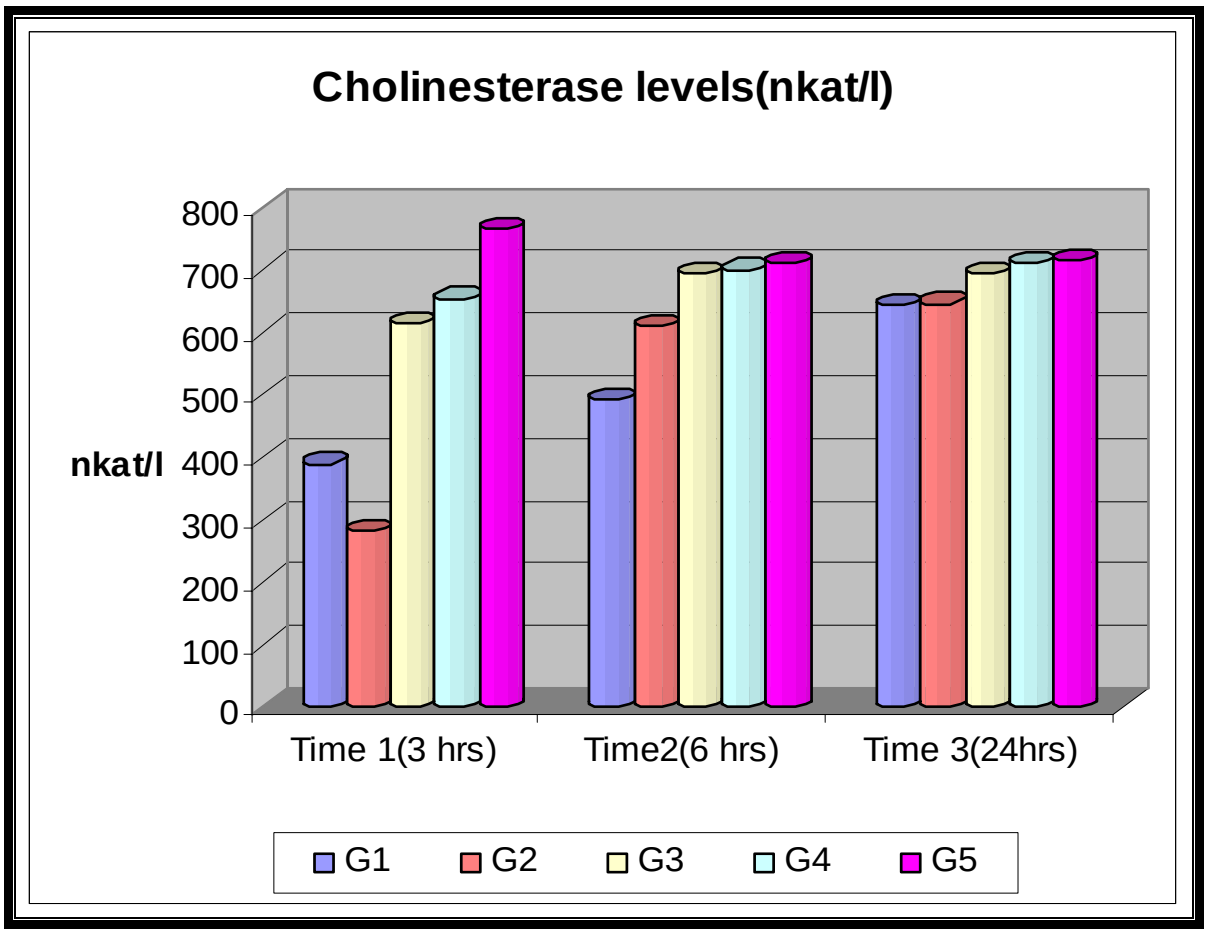


Fig. (10) Comparison between cholinesterase levels in rats treated with carbofuran, *Datura Stramonium* seed extract and carbofuran and Atropine sulphate and carbofuran

G1 = (carbofuran10mg/kg):

G2= (*D. stramonium* seed extract 5mg /kg body weight and carbofuran10 mg/kg)

G3= (*D. stramonium* seed extract 7.5mg /kg body weight and carbofuran10 mg/kg)

G4= (Atropine sulphate 17 mg /kg body weight and carbofuran10 mg/kg)

G5= (control)

increased in group 1 (carbofuran) and group 2 *D. stramonium* 5 mg/kg and carbofuran).There were no significant changes in levels of urea, creatinine and cholinesterase in the rest of the groups. Total protein, albumin and globulin were significantly decreased in group1 (carbofuran) when compared to the control group.

2.2 Treatment of malathion induced toxicity in rats with *Datura stramonium* seed extract:

2. 2.1 Clinical signs:

There were no obvious signs at the first half an hour of the experiment, except dullness and slow movement, for the four groups. After two and half hours, rats were provided with water and food. The treated groups 2, 3and 4(groups treated by the plant extract and Atropine sulphate) began to take their water supply normally, but remained unappitante. The untreated group (malathion group showed no response to the presence of water. Three hours later, (after one hour from the second dose), all the groups provided with food. Only, the treated groups started to take their food. After twenty –four hours the color of feaces, of the untreated groups (malathion group) changed from black to grayish and some white.

3.2 Pathological changes:

There was significant difference in the color of the liver (pale color) between the treated groups (2,3and 4) and the untreated group. Group 1, the untreated group (malathion group) had pale liver with hemorrhages, the heart, spleen, brain, and kidneys were characterized by severe congestion and hemorrhages. In group2 (Treated by *D. stramonium* 5 mg /kg and malathion) showed one rat distended stomach, severe congestion in the heart, spleen with fatty

changes in the liver and scattered spots of necrosis in it's upper surface. In group 3 (treated by *D. stramonium* 7.5 mg/kg and malathion) showed one rat with distended stomach, fatty changes and congestion in the liver and kidney, no significant changes in the heart and brain. Group 4 (treated by Atropine sulphate and malathion) showed distended stomach with gases in three rats. The liver is slightly congested. The kidney showed fatty changes. No significant changes, in the heart and brain.

3.3.3 Histological changes:

Histopathological changes in livers in groups 1, 2, 3 and 4 were showen in (fig 11, A-D).In group1 the untreated group (malathion group), there were congestion, and generalized necrosis, in group 2 treated by *D.stramonium* 5 mg/kg and malathion the histopathological changes in the liver were congestion in the central vein, fatty change and centrilobular necrosis. The liver in-group 3 had fatty change and slight necrosis of the hepatocytes. In group 4 (treated by Atropine sulphate) the liver had slight congestion, fatty change and slight necrosis.Other organs also showed different pathological changes. In group1, in the heart, there was lymphocytic infiltration. The kidneys were severely congested; with lymphocytic infiltration, and severe necrosis of the cortical and meddullary tubules plus necrosis the glomeruli. The spleen showed severe congestion. The brain had severe leucocytic infiltration.In group 2 (treated by *D.stramonium* 5 mg/kg and malathion the heart, kidney and spleen was severely congested with lymphocytic infiltration. The brain had mild infiltration. In group3 (treated by *D.stramonium* 7.5 mg/kg), the heart was slightly congested. The kidneys changes were lymphocytic infiltration, shrinkage of glomeruli, haemosidrosis. The brain showed no significant histopathological changes

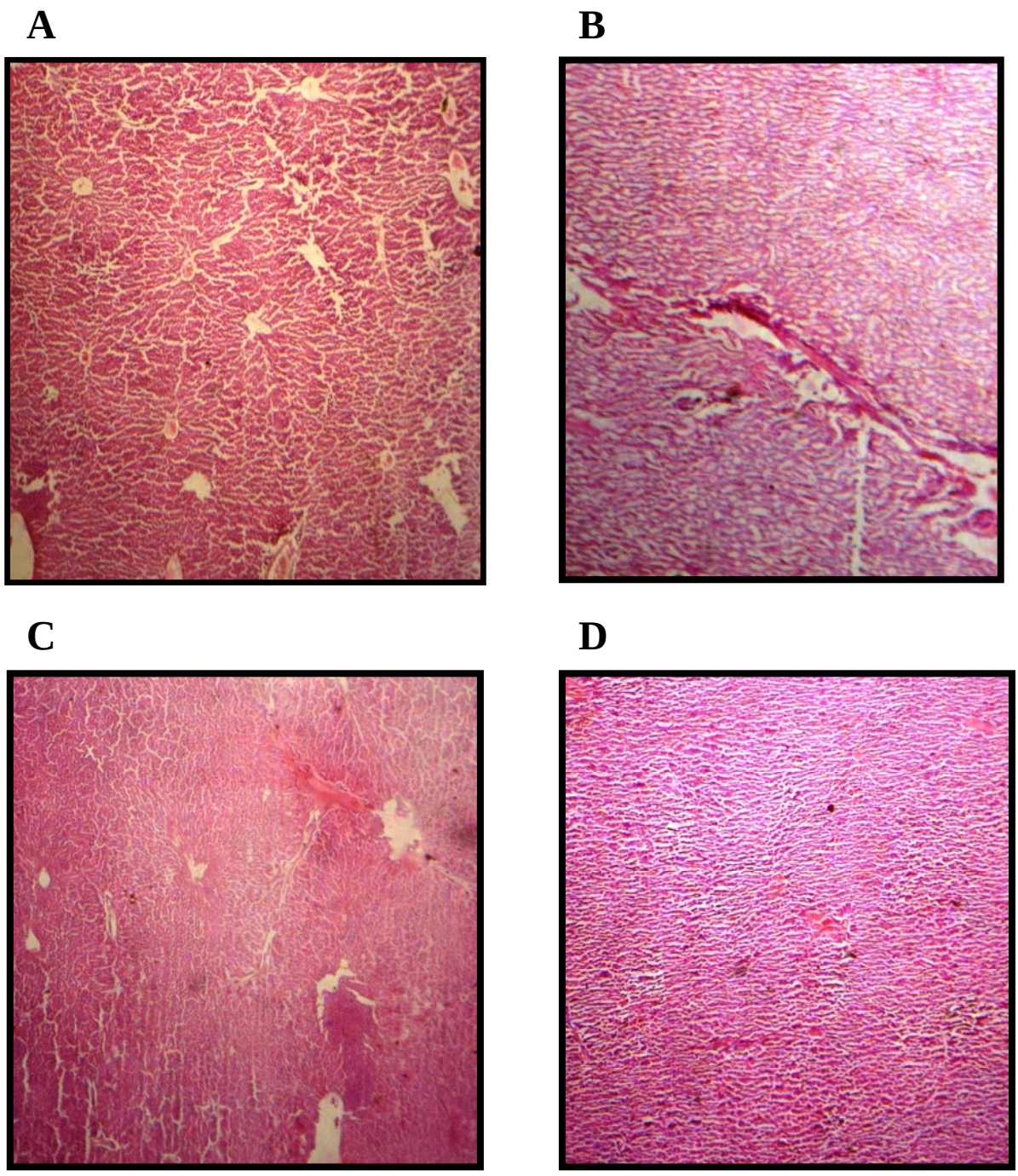


Fig (11) Sections of livers of rats treated with malathion , Atropine sulphate and *Datura Stramonium* seed extract 5mg and 7.5 mg /kg body weight seed extract 5mg and 7.5 mg /kg body weight.

A- Congestion, and generalized necrosis, in a liver of rat dosed malathion 500 mg/ kg

B- Congestion in the central vein, fatty change and centrilobular necrosis in a liver of rat dosed *D. stramonium* seed extract 5mg/kg and malathion 500 mg/ kg.

C- fatty change and slight necrosis of the hepatocytes in a liver of rat dosed *D. stramonium* seed extract 7.5mg/kg and malathion 500 mg/ kg.

D- Slight congestion, fatty change and slight necrosis in a liver of rat dosed atropine

Group 4 (treated by Atropine. The heart showed no histological changes. The kidneys changes were lymphocytic infiltration, shrinkage of glomeruli, with fatty changes of the meddullary tubules. The spleens showed slight haemosidrosis. The brain showed no significant histopathological changes.

3.4 Changes in the serum constituents:

Table(2) and (fig 12-15) shows the changes in serum constituents of rats post-treated by Datura stramonium seed extract (two doses 5mg/kg b.wt/ rat and 7.5 mg/kg b.wt/ rat) with regard to a reference drug –Atropine sulphate. After half an hour from the administration of the first treatment (by *Datura stramonium* seed extract and atropine) the activities of ALT increased significantly in group3 (malathion 500mg/kg/body weight and *D. stramonium* seeds extracts at a dose of 7.5mg/kg /body weight) , group 4 (malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight) and group 1 (only malathion 500 mg /kg/ body weight).The ranges of AST and urea, increased significantly in all the groups but were highly increased, in group 1 (malathion 500mg /kg/ body weight).No significant increase in creatinine level. The level of cholinesterase,was significantly reduced in the four groups when compared to the control group .ALP was increased in group 1(malathion 500 mg/kg /body weight), group 2 (malathion 500mg/kg /body weight and *D. stramonium* seed extract 5mg /kg/body weight) and group 3(malathion 500 mg /kg /body weight and *D. stramonium* seed extract 7.5mg /kg/body weight).Total protein, albumin and globulin showed no significant changes when compared to the control.

After half an hour from the second treatment (by *D. stramonium* seed extract and Atropine sulphate) the activities of ALT, AST and ALP and urea increased significantly in group 1(malathion 500 mg/kg /body weight) and

slightly reduced in the other groups. These groups are not significantly different. No significant increase in creatinine level. The level of cholinesterase was significantly reduced in group1(malathion 500 mg /kg /bodyweight) and group2 (malathion 500mg/kg /body weight and *D. stramonium* seed extract 5mg /kg/body weight), when compared to the control group. Total protein, and globulin showed significant reduction in group4 (malathion 500mg/kg/body weight) when compared to the control).

After twenty- four hours, the activities of ALT, AST, ALP urea and creatinine increased significantly in group1 (malathion 500mg/kg/body weight) while the level of cholinesterase is significantly reduced. Group4 (malathion 500mg/kg/body weight and Atropin sulphate) and group3 (malathion 500 mg /kg /body weight and *D. stramonium* seed extract 7.5mg /kg/body weight) showed normal levels of ALT, AST and ALP when compared to the control group, and the level of cholinesterase was significantly increased in these groups. Group 2 showed a reduction in the level of cholinesterase and normal levels of ALT and ALP, while the level of AST was significantly increased when compared to the control.

Total protein and albumin showed significant reduction, in group1 (malathion500mg/kg/bodyweight) and group2(malathion 500 weight and *D. stramonium* seed extract 5mg /kg /body weight) when compared to the control Globulin was significantly reduced in group1 (malathion 500mg mg/kg /body).whereas in group3 (malathion 500 mg /kg /body weight and *D. stramonium* seed extract 7.5mg /kg/body weight) and group 4 malathion 500mg /kg/bodyweight and Atropin sulphate) ,the level of total protein and albumin were not significantly reduced.

Table 2 Analysis of variance and Average (mean ±SE) values of serum constituents of rats post-treated with *Datura stramonium* seed aqueous extracts and intoxicated with malathion:

(TIME) After half an hour from the first treatment dose

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/dl)	Globulin(g/dl)	Albumin(g/dl)	Urea (mg/dl)	Creatinine(mg/dl)	Cholinesterase(nkal)t
F value	3.43*	1.57*	2.99*	1.08	1.07	3.25	3.61*	3.27	9.26*
G1	51.0± 20.5 a	286.2±2.5.a	200.4±17.7 b	7.2 ±0.3a	3.2±0.4a	3.5 ±0.1ab	54.0±3.1a	0.620.1a	159.33 38.7 cd
G 2	45.80±28.4ab	220.8±ab4.0b	197.2±27.3 b	7.3±0.3 a	3.7 ±a	3.3 ±0.1b	47.8 ±3.3bc	0.62a	126.67 d
G 3	51.0011.6±a	227.0±ab4.7b	182.0±26.6 b	7.9±0.2 a	3.5±0.1a	3.2±0.2 b	42.8±3.0 bc	0.72±0.02 a	361.048.7 ab
G4	52.00±19.1a	242±ab2.8b	118.6±8.9a	6.80±0.5a	2.7 ±0.5a	3.6±0.2ab	50.6±3.7 bc	0.880.1 a	363.6737.2 ab
5G	33.33 bc	178.33ab	110.0a	7.6 a	4.1± a	4.13a	38.67c	0.83 a	477.5 a

(TIME) After half an hour from the second treatment dose

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/dl)	Globulin(g/dl)	Albumin(g/dl)	Urea(mg/dl)	Creatinine	Cholinesterase(nkal)t
F value	**5.87**	**5.72**	**5.07**	**3.23**	**4.31**	**3.19**	**1.187**	**1.95**	**16.08**
G1	82.4 ±15.2a	293.80 ±15.1a	196.2±5.3a	5.8±0.2 b	2.4±0.3b	3.16±0.3 b	48.80 ± 5.7a	0.8±0.1 ab	277.00 ±28.7c
G2	42.6±31.2 b	230.0 ±5.2 b	137.60 ±18.1b	6.9 ± 0.1ab	3.6±0.3a	3.2 ±0.1ab	47.80±3.3a	0.6±0.1 b	291.67±30.4 c
G3	47.8±10.2b	219.00±3.4 b	115.80±20.5b	6.84±0.3 ab	3.2±0.2a	3.52±0.3ab	40. 0± 8.9a	0.9± 0.9a	351.67±40. 2 ab
G4	50.2±b9.5b	190.8 ±4.3b	117.00±10.5 b	7.00±0.6a	3.78 ±0.1a	3.1 ±b	50.25 ±a	0.72±0.3 ab	394.67 ±22.1ab
G5	29.75±4.7b	173.75 ±1.7 b	111.25±7.7 b	7.62±0.2 a	3.6±0.3a	4.05±0.1 a	38.25 ±0.6a	0.75±0 ab	486.00±103.5a

(TIME) after twenty-four hours

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/dl)	Globulin(g/dl)	Albumin(g/dl)	Urea (mg/dl)	Creatinine	Cholinesterase(nkal)t
F value	4.69	8.14	17.23	5.46	4.89	6.63	1.58	3.72	8.20
G1	91.40 ±28a	317.60 ±68a	210.60 ±10.9a	4.8 ±0.7c	1.6 ±0.6b	2.72±0 b	62.60±5.8b	0.94±0.2 a	216.67±10.3 b
G2	45.60±23.7 b	257.4±5.5 b	110.20 ±15.2b	5.80 ±0.4bc	2.8±0 a	2384±0.2b	48.20±5.6 a	0.5±0.6 c	253.00±36.5 b
G3	38.00 ±2.07 b	218.4±5.6bc	105.60 ±13.2b	7.24 ±0.6ab	3.5±0.2 a	3.68±0.4a	40.20±6.3a	0.8 ±0.1ab	353.00 ±72.6a
G4	35.00± 8.3b	188.80± 1.2c	106.60±5.2 b	6.18 ±0.2abc	2.84±0.4 a	3.34 ±0.1ab	40.40± 3.8 a	0.58±0.1 bc	370.33±57a
G5	29.75±3.8b	173.75±1.7 c	105.60 ±7.2b	7.650 ±0.2a	3.85±0.1a	3.97±0a	37.75±0.6 a	0.8±0.1ab	485.67±45 a

G1=only Malathion 500mg/kg/body weight.
G2=Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight
G3= Malathion 500mg/kg /body weight and Datura seed extract 7.5mg /kg/body weight.
G4 =.malthion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight.
G5=Control

Means with the same letter are not significantly different (.P> 0.05)

*At (4, 20) degrees of freedom. ** 5%

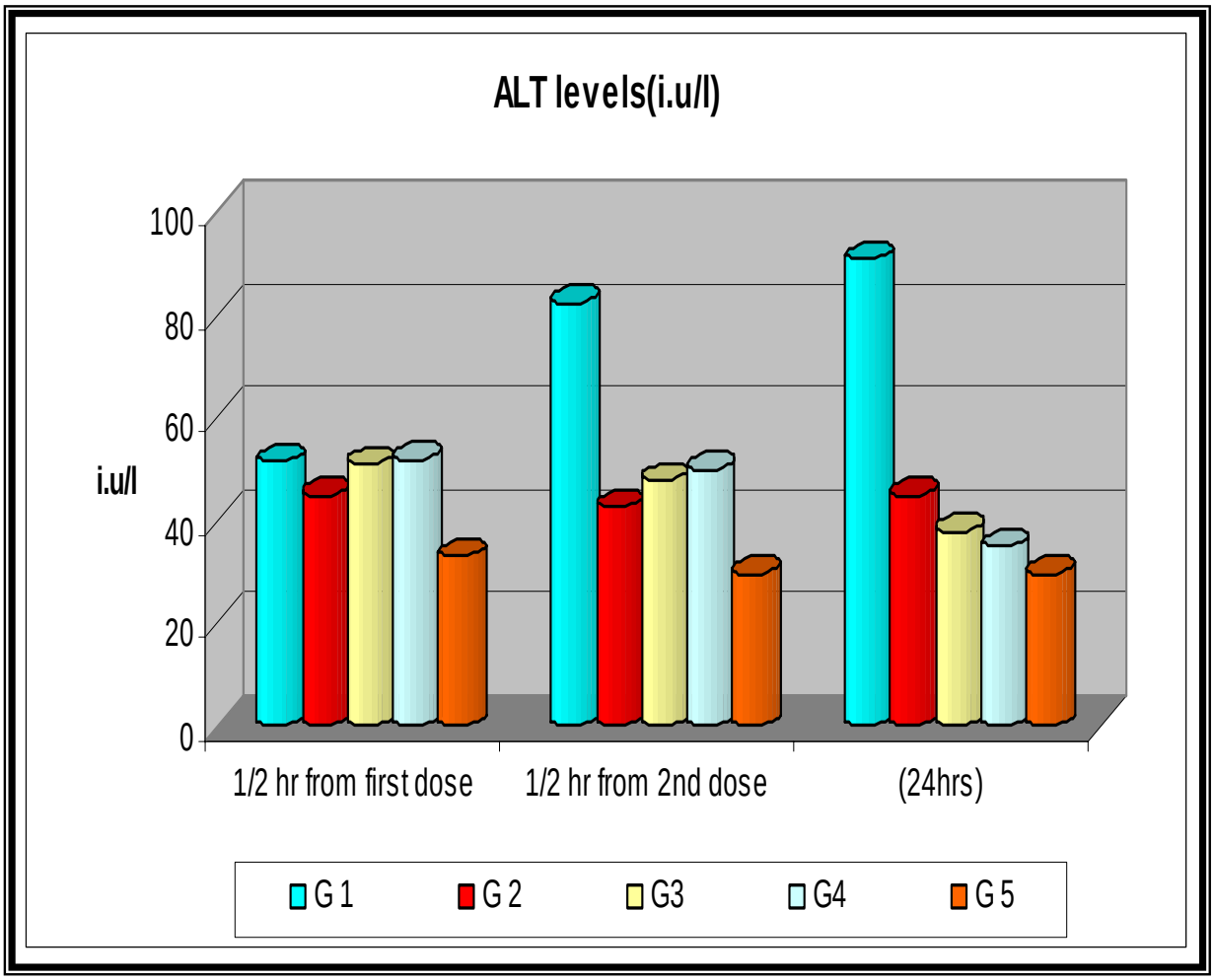


Fig. (12). Comparison between ALT levels in rats treated with malathion, *Datura stramonium* seed extract and malathion and Atropine and malathion

G1= Only Malathion 500mg/kg/body weight

G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg /body weight

G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight

G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight

G5= Control

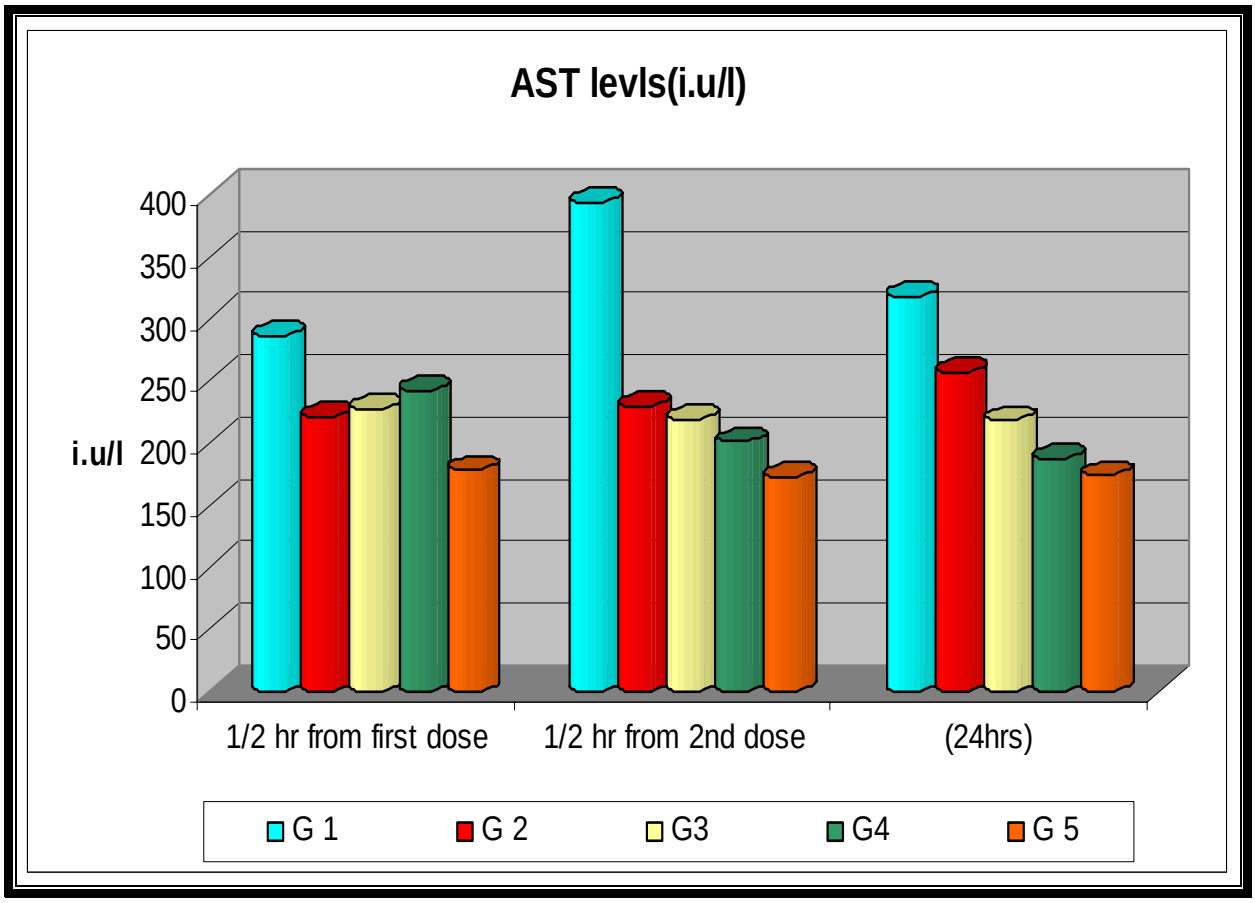


Fig. (13). Comparison between AST levels in rats treated with malathion, *Datura stramonium* seed extract and malathion and Atropine sulphate and malathion

G1= Only Malathion 500mg/kg/body weight

G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg/body weight

G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight

G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight

G5= Control

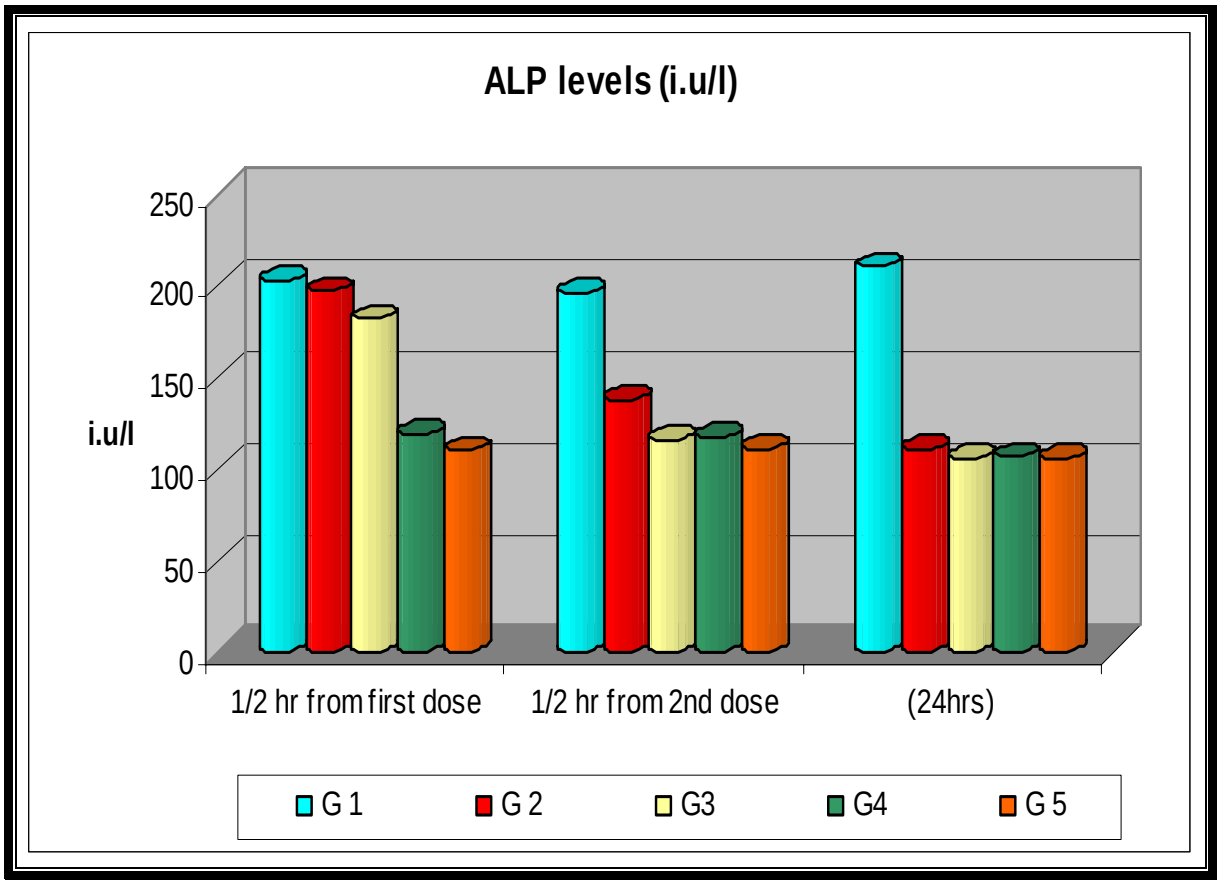


Fig. (14) Comparison between ALP levels in rats treated with malathion, *Datura stramonium* seed extract and malathion and Atropine sulphate and malathion

G1=only Malathion 500mg/kg/body weight.

G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg /body weight

G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight

G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight

G5=Control

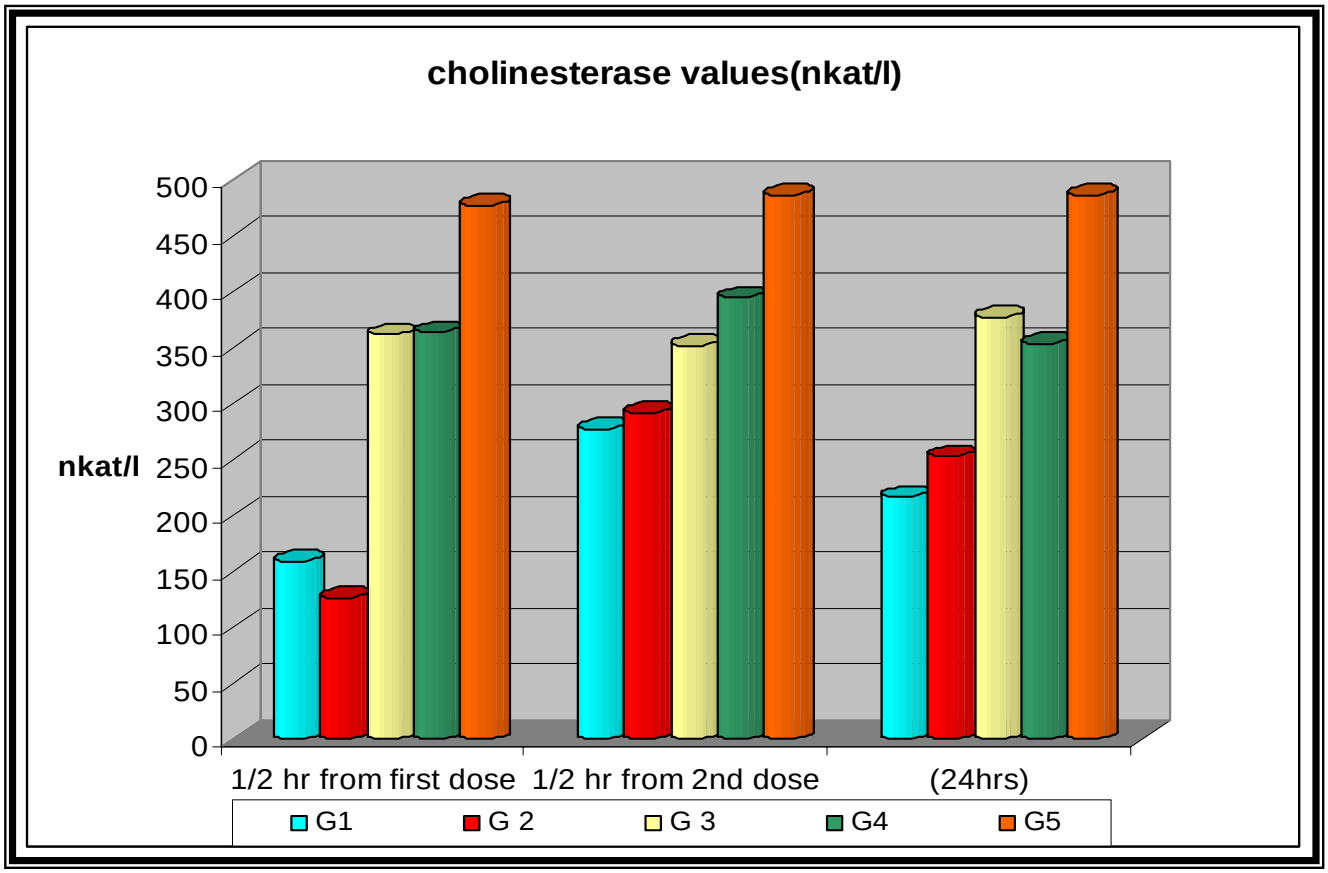


Fig (15) Comparison between cholinesterase levels in rats treated with malathion, *Datura stramonium* seed extract and malathion and Atropine sulphate and malathion

G1= Only malathion 500mg/kg/body weight

G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight

G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg /body weight

G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight

G5= Control

3 **Toxicity of *Datura stramonium* in white rats:**

3.3.1 Clinical signs:

There were no apparent signs of toxicity in the three treated groups.

33.2 Pathological changes:

At postmortem no pathological lesion were seen in group 1 (*D. stramonium* 7.5 mg/ kg) while in group 2 and 3(*D. stramonium*15 and 30 mg/ kg) showed severe congestion and hemorrhages in the liver, heart, and kidneys.

3.2.3 Histological changes:

No histopathological changes in the internal organs were recorded in group1 (*D. stramonium* 7.5 mg/kg). Group2 and 3(*D. stramonium* 15 and 30 mg/kg), the heart showed congestion and necrosis of the myocardium. The liver had severe necrosis, congestion and infiltration. The kidney showed dilation of the tubules with lymphocyte infiltration. The spleen had haemosidrosis. In the brain there was lymphocytic infiltration. The two groups had the same histopathological changes with lesser severity in group2 (*D. stramonium* 15 mg/kg). (Fig. 16)

3.2.4 Changes in the serum constituents:

Table (3) shows the changes in serum constituents of rats treated with *D. stramonium* seed extract at three level doses. The activities of ALT, AST and ALP increased significantly in group2 (*D. stramonium* seed extract 15mg /kg body weigh) and group 3 (*D. stramonium* seed extract 30 mg/kg body weight). Creatinine level was significantly increased in group 3 (*D. stramonium* seed extract 30 mg/kg body weight) when compared to the control group.

A **B**

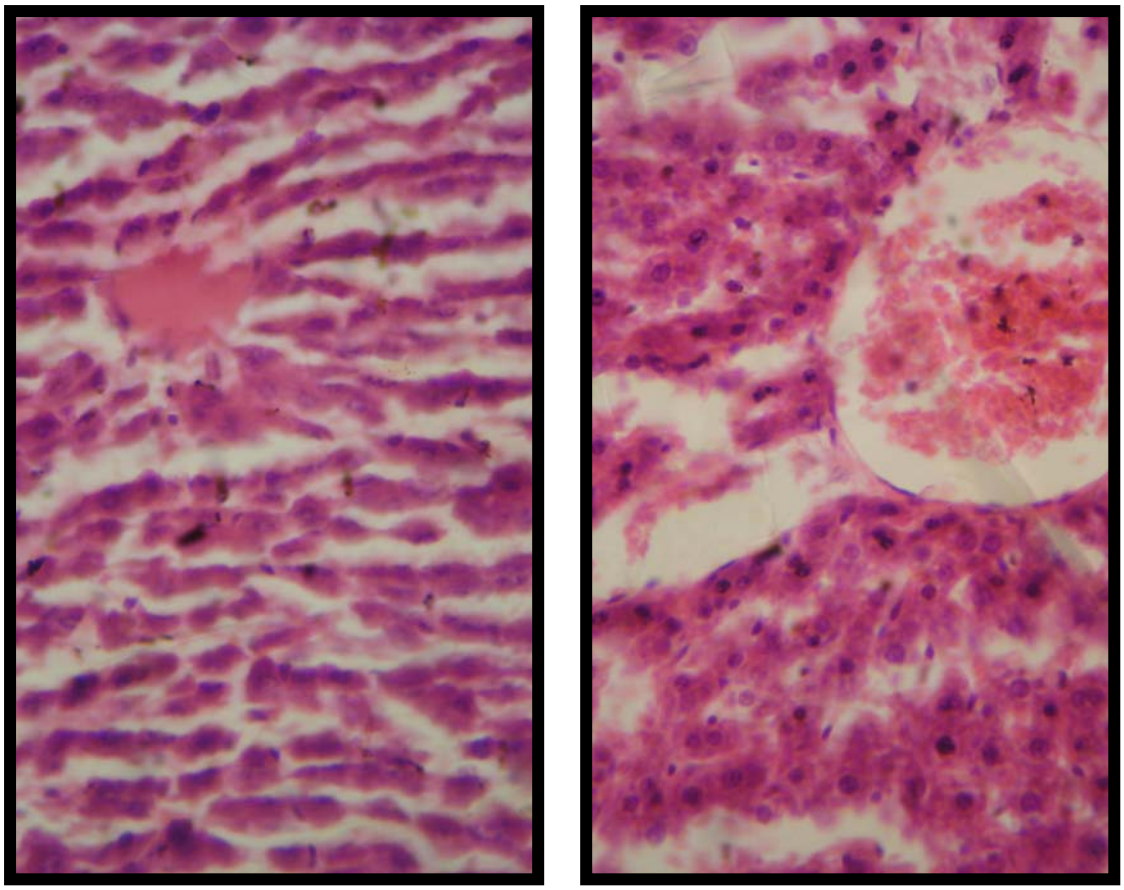


Fig.(16) Sections of livers of rats treated with *Datura stramonium* seeds extract.

A- Necrosis, congestion of the central vein in a liver of rat dosed *D.strramonium* seed extract 15 mg/kg

B- Severe necrosis, congestion of the central vein in a liver of rat dosed *D.strramonium* seed extract 30mg/kg

Table 3: Analysis of variance and average (mean ±SE) values of serum constituents of rats treated by *Datura stramonium* aqueous extracts

GROUPS	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	Albumin (g/dl)	Urea(mg/dl)	Creatinine(mg/dl)
F value**	21.03*	3.31*	2.26*	0.39	1.03	5.67*
Group 1	19.25±4b	86.00±1.5 b	39.75±0.5a	1.70±0.7a	.50a	0.15±b
Group2	20.75±7.8 b	131.00±3.0ab	45.00±1.4b	1.70±0.3a	26.25± 0a	0.22±1b
Group3	96.67±22 a	156.67±1 a	48.25±3.7b	2.06±0.3a	29.00±0a	0.36± 4.6a
Group4	14.50±29.6 b	72.00±17.6 b	40.50±1.2a	1.10±0.4a	22.0±0.3a	0.20±0.5b

G1=*D.strramonium* seed extract 7.5mg /kg body weight.

G2= *D.strramonium* seed extract 15mg /kg body weight.

G3= *D.strramonium* seed extract 30 mg/kg body weight.

G4= control

Means with the same letter are not significantly different (.P> 0.05)

*At (4, 12) degrees of freedom. ** 5%

CHAPTER FOUR
DISCUSSION

The present study has been an attempt to determine the efficacy of *D. stramonium* seed extract as an antidote to certain toxicities of insecticides (organnophosphurus and carbamate).

In this study the use of *D. stramonium* seed extract as protective agent in carbamate poisoning revealed that, the levels of ALT, AST and ALP at the first three hours in the groups treated by the plant extract (7.5 mg) and that treated by the drug Atropine, were likely similar to the control group, while the levels of these enzymes were significantly high, in the untreated group.
Case (1980) made an evaluation on the possible differences in brain and erythrocyte cholinesterase activity in adult and juvenile rats of both sexes following acute exposure to carbofuran. Examination of the optimal sampling time for evaluating maximum erythrocyte cholinesterase depression showed that approximately 30 minute following acute administration was the optimal in both sexes in adults and juveniles. The data showed recovery was almost complete four hours following acute carbofuran treatment. Complete recovery was noted at the 24-hours interval. It was quite evident that complete recovery of all enzyme depression was attained within one day.
While this study showed that the treatment with the extract and Atropine sulphate decreased the level of the enzyme cholinesterase compared to untreated group, before the four hours mentioned in the previous mentioned study. There were almost, complete enzyme recovery, in three hours time.
The histopathological changes in the liver, heart brain and spleen were the same changes that seen in the two treated groups by the plant extracts (7.5 mg/kg) and

that treated by the drug (Atropine sulphate). These results indicate that the dose of *D. stramonium* seed extract 7.5 mg/kg body weight, have almost the same effects of Atropine sulphate in treatment of toxicity.

The histopathological changes seen in the treated groups (by the plant extracts (5 mg/kg) and the untreated group were almost the same. After twenty –four hours the levels enzymes ALT, AST and ALP and the concentration of urea and creatinine were similar to the control group with slight increase in level of AST in the untreated group. The concentration of total protein, albumin and globulin were significantly decreased in group1 (carbofuran) when compared to the control group. Almost complete recovery of most of the enzymes was retained in twenty-four hours compared to three hours time recovery of the other two groups (Atropine and *D. stramonium* 7.5 mg/kg). These preliminary pretreatment suggest the possibility of using the extract as a post treatment antidote for carbamate poisonings.

The study for evaluating the use of *D. stramonium* seed extract as a post treatment in malathion poisoning had showed significant increase in ALT during the first half an hour which indicated tissue damage. This also recorded in study by (Murphy, 1966) that after single intraperitoneal or oral doses of malathion, trichlorofon or dioxathion in rats, an increase in the activities of liver tyrosine transaminase and alkaline phosphatase, as well as a decrease in the level of adrenal ascorbic acid were found. Further results of this experiment support the hypothesis that acute poisoning may produce metabolic alterations which are mediated through the pituitary-adrenal system.

After half an hour from the second dose, the levels of AST, ALT and ALP in group 2, 3 and 4(*D. stramonium* 5mg/kg, *D. stramonium* 7.5 mg/kg and

Atropine sulphate) were reduced and significantly different from the control group. After twenty four hours the levels of these enzymes in all the groups, reduced almost to the normal except for the malathion group. Group 2 (*D. stramonium* 5mg) had slightly higher enzymes levels than group 3 and 4(*D. stramonium* 7.5 mg/kg and Atropine).These results together with histopathological changes which recorded similar pathological lesions of group 3and 4(*D. stramonium* 7.5 mg/kg and Atropine sulfate) Indicate the effective action of the treatment.

The levels of total protein, globulin and albumin; which showed high reduction in malathion group and recorded that the levels of the metabolic indicators had lesser values when compared to group 3 and 4 (*D. stramonium* 7.5 mg/kg and Atropine), can pointed out another evidence of the extract protective effect.

There was apparent reduction in cholinesterase activity in all the groups during the first half an hour after the first dose and this was recorded by (Frawley *et al*., 1957), who study the simultaneous administration of malathion and ethyl pnitrophenyl thionobenzenephosphate (EPN) results in a potentiation of the cholinesterase inhibitory effect of malathion in the mouse, rat and dog. The level of this enzyme began to increase after twenty –four hour especially in group 3 and 4 (*D. stramonium* 7.5 mg/kg and Atropine) which means that the body began to eliminate malathion from its tissues.

The organophosphate induce reductions in dynamic lung compliance, arterial oxygen tension, increase in total pulmonary resistance, work of breathing and alveolar arterial oxygen gradient, were reversed by Atropine sulphate. Atropine may therefore, reverse changes in ventilation perfusion

inequalities resulting from uneven distribution of ventilation, caused by acetylcholine-mediated airway constriction (Slocombs and Robinson, 1987). This resemble other studies in antidotal activities in calves poisoned with intravenous dichlorvos, Atropine was shown to reverse the respiratory effects of the organophosphate (Likeux *et al.*, 1986). Atropine increases the survival of rats by reversing the action of acetylcholine at muscrinic receptors and minimizes air way constriction. While the extract of *D. stramonium,* has a long duration of effect. (Clancy *et al*., 2001)

The usual duration of anticholinergic effect following exposure to the *D. stramonium* extract is 12–48 hours. (Levy 1977, Chang *et al*., 1999 and Clancy *et al*., 2001)This long duration of clinical effect has potential advantage in the treatment of OP toxicity since the effect of most OP will be long. Patients treated with this extract may not require additional doses of antidote. *D. stramonium* cross the blood–brain barrier, and has central anticholinergic effects and antagonizes the peripheral muscarinic receptor. (Clancy *et al* 2001) The high concentrations of these anticholinergic alkaloids in the *D. stramonium* seeds make an extract of this plant a potentially useful agent for the treatment of organophosphorus toxicity.

In our study to determine the safety of the mentioned dose of *D. stramonium* seed extract 7.5 mg/kg body, and to evaluate the possibility of increasing this dose, the study showed that the level of ALT, AST and ALP were significantly high in the other doses 15, 30 mg/kg body weight.

As the measurements of ALT, AST and ALP enzymes renders a reliable assessment of liver damage (Gupita *et al* 2004); our study showed that increased level of this enzymes, in 15mg and 30 mg group indicates hepatonephropathy changes. This result was correlated with the result obtained with the toxicity of some plants namely *Aristolochia brachteta* toxicity in goats (Barakat 1978; Barakat *et al*., 1978; Eldirdiri *et a.,l* 1987)

and resembles those lesions detected in goat intoxicated with *Capparis tementosa* (Siham *et al*., 1993), also the postmortem findings in the toxicity of *datura stramonium* in sheep and goats (El Dirdiri *et al*., 1981). The normal levels of the same enzymes revealed no toxicological evidence of the dose used (7.5 mg of *Datura stramonium* seeds extract) together with the result of histopathology indicated the safety effect of this dose. At the same time the possibility of increasing the dose may be toxic to the animal.

Conclusion:

1. The protective action *D. stramonium* seed extract against the two insecticides tested was dose dependant.
2. Treatment with *D. stramonium* seed extract has a significant protective action in a rat model of organophosphorus and carbamate poisoning.
3. The dose of *D. stramonium* seed extract 7.5mg/ kg body weight/ rat is safe and the possibility of increasing the dose could be toxic to animals.

Suggestions for future work:

1. Evaluation of the plant extract as antidotal agent in other species where the symptoms of toxicity is apparent.
2. Estimate the benefit of the dose repetition according to specific time interval.
3. Evaluation of any toxicity produced by other extracts of the plant.
4. Evaluation of *D.stramonium* against other commonly used organophosphorus and carbamate compounds.
5. Future studies should evaluate the effect of other routes of administration of the extract such as via the intragastric, oral , mucosal or sublingual route.

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